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Photosynthate translocation

Robert Stanley Gage
Iowa State College

PHOTOSYNTHATE TRANSLOCATION

by

Robert Stanley Gage

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Approved:

Signature was redacted for privacy.
In Charge of Major Work

Signature was redacted for privacy.
Head of Major Department

Signature was redacted for privacy.
Dean of Graduate College

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INTRODUCTION

Few aspects of plant physiology have a longer history or a more voluminous literature than that associated with the movement of materials within plants but, in spite of this, there is no aspect in which so little agreement has been reached. The term translocation has been applied to the movement of all organic and inorganic materials between different regions of a plant, in wide limits of space and time. Its usual connotation, however, implies movement of metabolites in phloem tissue as distinct from movement of solutes with the transpiration stream of the xylem. Translocation studies have been made using a wide variety of natural and artificial substances. Early experiments were restricted to measurements of sugar gradients in plant sections and examination of phloem exudate; or to the use of artificial indicators like dyes and viruses. With recent advances in biochemistry - particularly in radiobiochemistry - measurements have been extended to a wide variety of metabolites. There is general agreement that normal movement may occur at rates which are far in excess of those to be expected by simple diffusion and that transport is confined to phloem tissue. The minute sieve cells with their linear arrangement into sieve tubes are suspected of being the conducting channels. Several translocation mechanisms have

been proposed but none appears to satisfy the empirical findings.

Extensive reviews covering all aspects of plant translocation have been written by Curtis (20) in 1935; Curtis and Clark (21) in 1950; Crafts (16) in 1951; Vernon (60) in 1951 and Arisz (1) in 1952. In the historical survey that follows, an attempt will be made to present only the major contributions to photosynthate translocation with greatest emphasis on the period since 1951.

There is general agreement that sucrose is the chief photosynthetic product moved in plants. Early workers like Huber et al. (33) working with trees; Mason and Maskel (43), with cotton; Engard (25), with raspberry; Leonard (40), with sugar beets and Loomis (41), with corn came to this conclusion from chemical analyses of phloem exudate and tissue sections. Vernon and Aronoff (61) applied the techniques of radiochemistry and chromatography to extracts of soybean stem sections and gave the first conclusive evidence that sucrose is the main material transported to other parts of the plant. Smaller quantities of fructose, glucose and raffinose were also observed as well as detectable amounts of some organic and amino acids. They also demonstrated that material entering the stem moves predominantly in two directions. A small fraction moves upward toward the growing point but the greater amount goes downward toward the root. The distribution of tracer in the stem, in short-time

experiments decreases logarithmically from the entrance petiole. These results have since been confirmed by Nelson and Gorham (47), also working with soybeans; Swanson and El-Shishiny (56), with grape and Ziegler (65), with trees. Zimmermann (66) has found that appreciable amounts of raffinose, stachyose and possibly verbascose are transported in some tree species. He makes the interesting observation that all the sugars identified were closely related and include the sucrose moiety in their structure.

The evidence that plant foods travel out of the leaf and downward through phloem tissue is largely of an indirect nature. Early investigators like Zacharias (64), Lecomte (39) and Fischer (26) came to this conclusion when it became apparent that sugars moved independent of transpiration water. Mason and Maskel (43) and Curtis (20) performed many girdling experiments in which a ring of bark, containing the phloem, was removed leaving the xylem intact. It was amply demonstrated that sugars accumulated at the ring. Biddulph and Markel (7) and Stout and Hoagland (54) successfully separated the phloem and xylem of cotton stems with cylinders of waxed paper. They observed upward movement of P^{32}, absorbed by roots, only in the xylem but application to the leaf resulted in downward movement only through the phloem. More direct evidence has come recently from the technique of autoradiography. Biddulph's autoradiograms of Red Kidney bean stems, following foliar applications of P^{32}

and S^{35} (8), show localization largely in the phloem region. Thaine and Walters (58) have developed a technique for autoradiography of fresh soybean stem sections, following $C^{14}O_2$ administration to leaves. Their initial photographs show beta-tracks within a sieve tube but also in the contents of phloem fibers.

The amount and rate of carbohydrate translocated have been estimated by many workers. Mason and Lewin (42) calculated that 43 gm of organic material moved into the tuberous root of yam, over a four week period. Assuming an average cross section for sieve tubes of 0.01 cm^2 and that the carbohydrate moved in a 25 percent solution, an average linear velocity of 88 cm per hour would be necessary. Crafts and Lorenz (17) made similar calculations for cucurbit fruits and arrived at about 11 cm per hour, if the organic matter moved in the dry state, and 55 cm per hour, in a ten percent solution. On the further assumption that the material is restricted in its movement to the cytoplasm, a velocity of 5 m per hour would be required. Huber (32) estimated that downward movement in the phloem of broad-leaved trees must range up to at least 100 cm per hour. The use of radioactive tracers has provided direct measurements of velocities. Vernon (60) estimated rates of about 95 cm per hour in soybeans during 20 minute translocation of C^{14}-labelled photosynthate, in so far as he was able to locate the activity front. Nelson, et al. (50) fed large activities of $C^{14}O_2$

to the primary leaves of young soybeans and found significant amounts in the root after 30 seconds, corresponding to a rate in excess of 10 m per hour.

These velocities of movement cannot be accounted for by any simple diffusion process and a number of translocation mechanisms have been proposed which have led to a good deal of experimentation and controversy. Two popular ones are the "pressure-flow" and the "protoplasmic-streaming" hypotheses.

The first was proposed by Munch (46) and was championed, in modified versions, by Crafts (12, 14) and others. It assigned to the sieve tubes the role of passively carrying translocate in solution. It assumed that high osmotic pressures in leaf, parenchyma cells forced solution through plasmodesmata into the sieve cells and thence through plasmodesmata into the receiving cells, where the concentration was lowered due to utilization of the solute. Due to the semipermeable nature of the sieve cell membranes, the transported solution was envisaged as flowing along a pressure gradient. Support for this mechanism was found by Munch (46), Dixon (23) and Crafts (12, 15) when it was found that sap, containing high sugar concentrations, will exude for considerable lengths of time from cut phloem. Sieve tubes, even in wilted plants, commonly appeared to be under considerable turgor pressure. Bennett (5) has shown that certain viruses appear to move through the phloem with carbohydrate. Translocation of 2,4-dichlorophenoxyacetic acid also is closely correlated

with photosynthate movement as shown by Mitchell and Brown (45) and others. Because the pressure-flow hypothesis is formulated on known, elementary, hydrodynamic principles, it has been relatively easy to examine critically. Crafts (11), assuming Poiseulli-flow and estimates of sieve tube and sieve plate pore dimensions, calculated that impossibly great pressures would be necessary in order to carry sugars in solution at the known rates. More recently, however, Crafts (16) has proposed the existance of 200-300 A micropores in the sieve plates which would offer less resistance to flow. Clements (10) measured daily increments in dry weight of the fruits of Kigelia africana and by correlating this with phloem dimensions and sugar concentrations, estimated that 5 liters of solution must traverse the peduncle each day. These volume increments were not reflected in the fruit growth and the water could not be transpired through the heavy cuticle. He was able to slit the bark longitudinally and sever the xylem. No return flow of water was observed during normal growth. Other objections to the pressure-flow hypothesis have been noted by workers showing that different materials may move simultaneously in opposite directions, or in the same direction at different rates. Chen (9) found a slow, bidirectional flow of P^{32} and C^{14} in the stem section of tomatoe, between two leaves to which the tracers had been applied individually. The translocation of fluorescein can be quite independent of that of carbohydrates and nitrogenous

compounds [Palmquist (51), Schumacher (52)]. Swanson and Whitney (56) analysed the distributions of two tracers applied simultaneously to kidney bean leaves. K^{42} and P^{32} were distributed in about the same ratio down the stem but the activity ratios of Cs^{137} and P^{32} varied widely. P^{32} moved into roots and leaves more readily than Cs^{137} which, in turn, was more mobile in this respect than K^{42}. Leonard (40) and Loomis (41) have drawn attention to the apparent polar movement of sugars from parenchyma to phloem, against concentration gradients. Fruits commonly contain higher sugar concentrations than the tissue from which the sugar comes. Curtis and Clark (21) point out that this may be explained in part if one assumes that sugars move into the phloem cells in hexose form and there are converted into sucrose, the sieve tube membranes being impermeable to sucrose.

The second popular hypothesis, that of protoplasmic-streaming, was suggested by De Vries (22) in 1885 and has been supported, in recent years by Curtis (20, 21). A further elaboration is the "active-diffusion" hypothesis of Mason and Phillis (44). The basic assumption is that solute molecules are carried from one end of the sieve tube to the other by the rotating protoplasm; and move across the sieve plates by diffusion or with interconnecting protoplasmic strands. Because of the unknown structure and energetics of the sieve tube plasm, this theory is difficult to examine

analytically and, in this respect, its proponents are safer from criticism. Support has come from in vivo, microscopic observations of cycling protoplasm seen in the large sieve cells of plants like cucurbits; although streaming from one cell to another has never been observed. Considerable work has been done to demonstrate that respiration is involved. Kursanov and Turkina (38) found respiration rates as high as 5 ml, O_2 per hour per gm of tissue in the vascular bundles of sugar beet and increased rates when sucrose or other organic substances pass through the conducting tissue. A depression of carbohydrate movement has been demonstrated, by lowering the temperature of stems and petioles, by Curtis (19), Swanson and Bohning (55) and Vernon and Aronoff (61); although Went (62b) interprets his petiole bleeding data in the opposite sense. Similar retarding effects have been observed by depriving stems and peticles of oxygen [Curtis (19), Mason and Phillis (44)]. Transport is hindered when the conducting tissue is exposed to metabolic inhibitors like KCN, 2,4-dinitrophenol and NaF [duBuy and Olson (24), Kendall (37a)]. Gauch and Duggar (28) and Sisler, et al. (53) postulate that a sugar boron complex is involved and have found accelerated movement of sucrose into cut petioles of tomatoe when boron was added to the fed solution.

A mechanism that has received less attention is one proposed by van den Honert (59) who has described an apparatus which demonstrates the rapid spread of surface-active

material over a water-ether interface. This also meets with the difficulty of operational definition since it would require a continuous, liquid-liquid, interface complex extending from one extremity of the plant to the other.

Attention has been directed, in recent years to critical, microscopic examinations of phloem cells. Currier, et al. (18) have demonstrated reversible plasmolysis of mature sieve cells in a wide variety of species; but question free passage of material across sieve plates which, in mature, presumably functional phloem, appear to be plugged with "callus". Hepton, et al. (29) examined sieve plates of Cucurbita under the electron microscope. Their photographs indicate that the sieve pores are completely filled with an electron-dense material that would provide an impenetrable barrier between sieve cells. They find no evidence for existence of the micropores proposed by Crafts (16). Frey-Wyssling and Muller (27b) made electron micrographs of the skeletal structure of differentiating Cucurbita sieve cells. They found interconnecting pores in the sieve plates and believe they are formed by the growth of a protoplasmic plug covering the area of the future pore. One-sided sieve plate areas were also found in sieve cells adjacent to companion cells but the latter did not cooperate in the production of interconnecting pores.

Within the past two years some critical experiments have been reported. Nelson and Gorham (48) have fed different

radioactive solutions through one primary leaf petiole of young soybeans. In short-time experiments, glucose and fructose moved rapidly past steamed sections of the stem, indicating movement outside of the phloem; while sucrose was blocked as would be expected from phloem movement. Nelson and Gorham (49) found that amino acids, applied in the same manner, moved at much greater rates than sugars, some moving preferentially into the opposite primary leaf. They also find greatly retarded movement in plants with chilled roots.

Biddulph and Cory (6) analyzed the distribution of three tracers applied simultaneously, in the form of THO, $NaH_2P^{32}O_4$ and $C^{14}O_2$, to the same leaf spot of Red Kidney beans. THO departed from the logarithmic distribution of P^{32} and C^{14} in the stem and was recovered in much smaller amounts, relative to the amount applied, as compared to the other tracers.

Horwitz (31) has provided an elegant and timely quantitative analysis of the consequences of a number of translocation theories. He finds reasonable theoretical agreement with the data of Biddulph and Cory (6), Swanson and Whitney (57) and Vernon and Aronoff (61) for mathematical models which assume mass movement of sieve tube fluid, through pipes with reversible and irreversible loss through the walls, and a time variable source of supply. Theoretical justification was also shown for long-term data with models involving

protoplasmic cyclosis and diffusion. By applying the criteria for Newtonian fluid-flow, he has shown that turgor pressures of the order of 20 atm would be required for "pressure-flow" of solution through micropores of 400 A dimensions. He was unable to justify the surface-flow hypothesis of van den Honert (59) on the basis of a mathematical model.

In order to define a problem within reasonable limits, it was decided to restrict this investigation to translocation of photosynthate as it occurs initially from its origin in the leaf to the rest of the plant. Vernon and Aronoff (61) did their classical work on the identification and distribution of photosynthetic products in the same laboratory, and kinetic studies on rates of movement were also initiated. This research is a continuation of their work.

At the time it was begun, it seemed advisable to examine photosynthate translocation from the following points of view:

I. The influence of meristematic tissue and other plant parts.
II. The fate of a nonmetabolite when introduced to the plant simultaneously with photosynthate.
III. The movement of water with photosynthate.
IV. The path of movement employing the high-resolution, autoradiography possible with tritium.

A good deal of the work involved the development of experimental techniques peculiar to each aspect. Accordingly, the thesis is divided into four sections, each complete with experimental methods, results and discussion.

PART I. GROWING POINTS, LEAF, STEM AND ROOT INFLUENCE

This section describes experiments designed to examine the influence of plant parts on short-period, photosynthate movement using two different feeding techniques. They consisted of measurements of the distribution of C^{14}-labelled photosynthate following excision of various plant parts and steam treatment of the stem

Experimental

Plant material

Soybeans (Glycine max, variety Hawkeye) were grown in the greenhouse at ordinary temperatures. During winter months, natural illumination was supplemented with about 1,000 ft-c using daylight fluorescence lamps and the photoperiod adjusted to about 14 hours. All plants were grown in soil except for Expt. 1,a where they were supported in vermiculite over jars containing Hoagland's culture solution (30) which was changed daily. They were moved to the laboratory about three weeks after planting when the first trifoliate was fully grown and the growing points were about one quarter expanded. Only healthy plants were used. In the double-feeding trials, two plants of identical growth and appearance were selected from the same planting pot. All plants were preconditioned to the laboratory environment for two or three

hours and any pretreatment was done at least one hour in advance where possible. Excision of leaf and stem parts was accomplished with a clean stroke of a razor. Root tips were removed by cutting with surgical scissors just past the region of cell elongation, while the root was immersed in culture solution.

C^{14} administration

$C^{14}O_2$ was administered to leaves in one of two ways. In Expt. 1,a, a method similar to that described by Aronoff (2), was modified so that two leaves could be exposed simultaneously to the same activity. A small area (about 1 cm^2 midway between the margin and mid-vein) on the under surface of the central, trifoliate leaf of each plant was sealed with vacuum grease to each arm of the Y-shaped vessel shown in Figure 1,a. A known amount of $C^{14}O_2$ was released from $BaC^{14}O_3$ with dilute $HClO_4$ in a separate, mercury-sealed, tygon, reaction tube. The gas was withdrawn with a hypodermic syringe and injected into the feeding vessel through the serum-vial stopper. The apparatus also contained a steel needle, sealed in glass, to facilitate uniform mixing with an external magnet.

For $C^{14}O_2$ administration to entire trifoliates, the same spherical chamber used by Vernon (60) was employed (Figure 1,b). The hemispheres were sealed with vacuum grease and the petiole with modeling clay. The leaf chamber was

connected by tygon tubing in closed series with the reaction flask (Figure 1,c) and a finger pump that circulated the gas at a rate of about 10 liters per hour. The treated leaves were illuminated with about 1000 ft-c of water-filtered radiation from a 150 watt, incandescent, spot lamp. $C^{14}O_2$ was first generated from $BaC^{14}O_3$, in the closed reaction vessel and then released to the system by opening the stop cocks.

In petiole feeding experiments, the plants were supported with stems horizontal and the petioles pointed downward. The leaf was removed under water, at its union with the petiole and the feeding tube, shown in Figure 1,d was slipped over the cut end. All but a small drop of water, at the petiole end, was removed from the tube; the petiole arm was sealed with modeling clay; the radioactive solution was added through the open arm and mixed and the open arm was sealed with a stopper.

Zero time, with both feeding techniques, was taken to be that time when the radioactive material was released to the tissue.

C^{14}-photosynthate assay

For Expt. 1,a, the roots were quickly blotted and the plants were autoradiographed by placing them against Kodak, X-ray, No-screen film, in a light-tight press which was

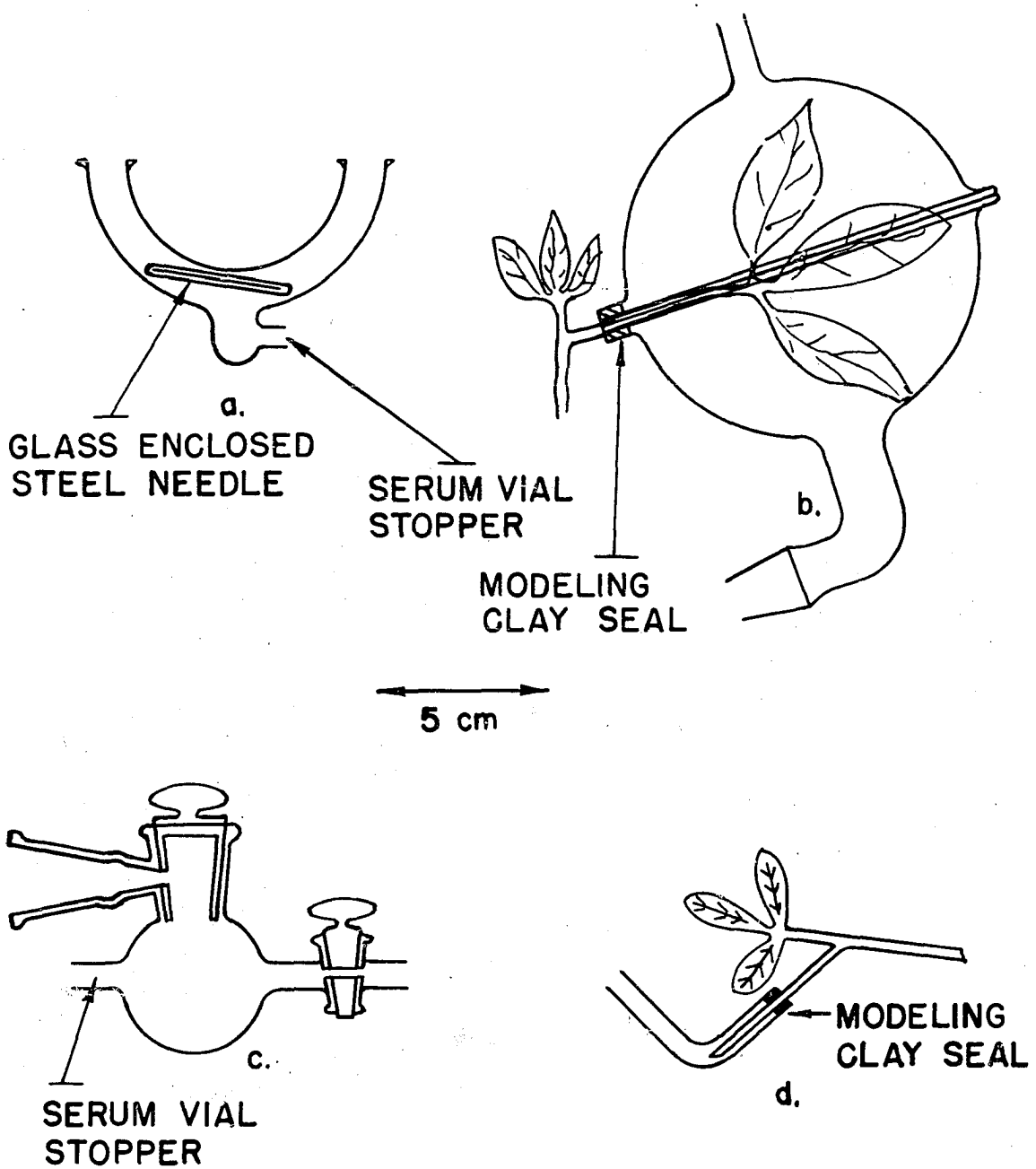


Figure 1. Apparatus for administering radioactive material

a. single leaf (two plants), b. trifoliate,
c. reaction vessel, d. petiole.

stored at -10° C. The film was processed, using standard developer, after a 14 day exposure.

In the remainder of the experiments, the plants were sectioned and the alcohol-soluble extracts were assayed, using Vernon's procedure (60), in the following sequence:

a) At the end of the feeding period, the stem was severed at its base and the plant was stripped of cotyledons, primary leaves, growing point, fed-trifoliate and fed-trifoliate petiole. The stem was then accurately divided into 2 cm sections. All portions were sealed in screw-top test tubes which were immersed in liquid nitrogen and then stored at -10° C. The sectioning and freezing procedure was accomplished in about one minute.

b) Leaf material was given three, 15 minute refluxes in 80 percent boiling ethanol. This extract was evaporated to a small, known volume under reduced pressure and an aliquot taken for counting. Stem sections were pulverized in a Ten Brock, tissue grinder. The homogenate was transferred quantitatively, in 80 percent ethanol, to a small centrifuge tube, brought to a boil and spun at low speed. The supernatant and those from two subsequent pellet elutions were combined and sampled as above.

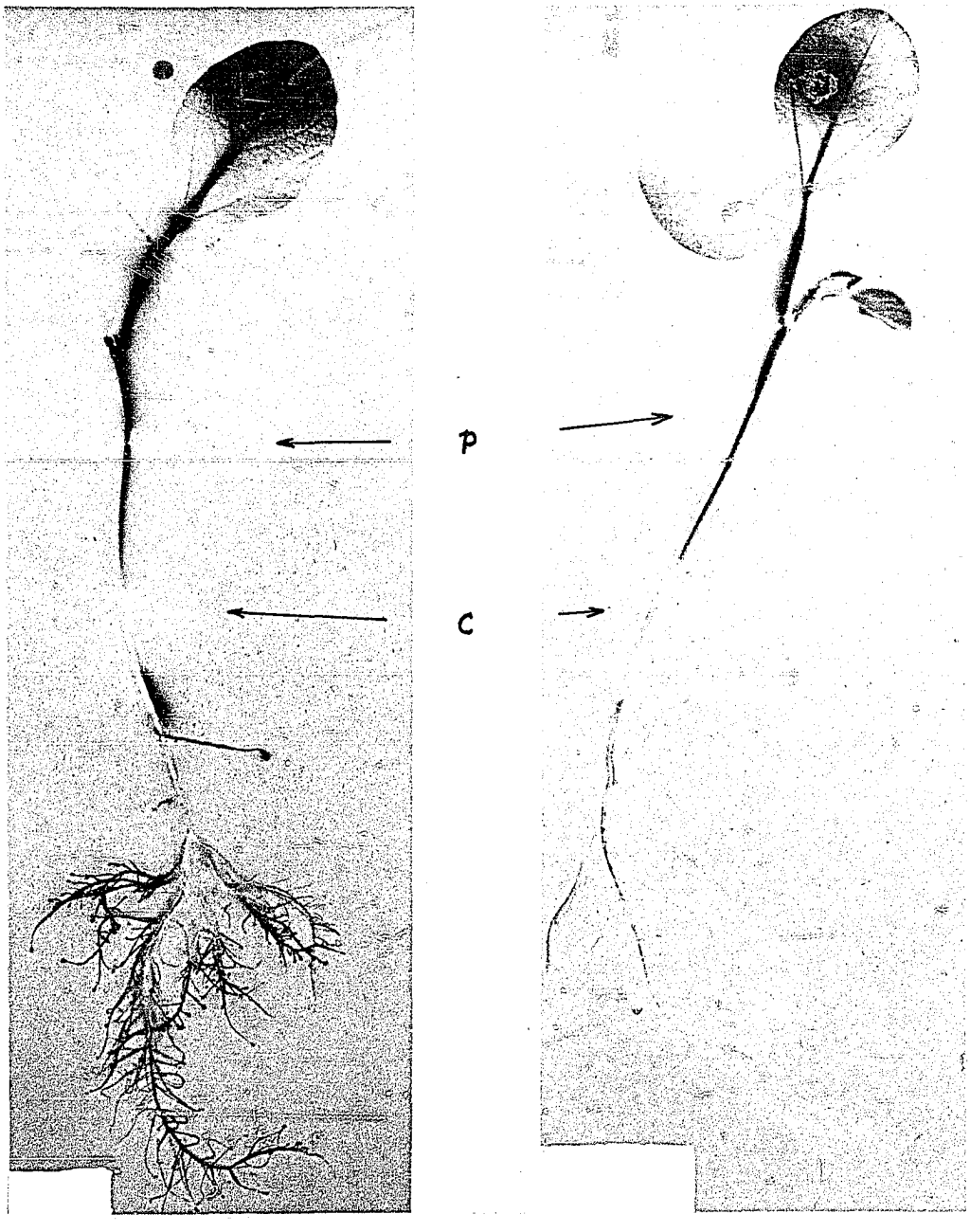
c) The aliquots were spread uniformly, in layers of infinite thinness, on frosted glass, counting plates and air-dried. C^{14} activity was measured in a Nuclear, Model D-46, windowless flow-counter operating on the geiger plateau. Sample

and background counts were taken for the length of time required to give standard deviations of plus or minus 3 percent.

Results

Expt. 1,a. Macro-autoradiography; 30 min, $C^{14}O_2$, leaf administration; root tips, growing point removed

Exploratory tests indicated that soybeans translocated autoradiographically detectable amounts of C^{14}-photosynthate when administered approximately 1 microcurie of $C^{14}O_2$ to a small area of a trifoliate leaf, significant darkening appearing in the roots after 20 minutes. Subsequently, 30 minute, double-plant experiments were performed to determine the effect of excision of meristematic tissue. No significant difference in distribution could be found, in three trials, between controls and plants that had root tips or growing-points removed. Figure 2 shows the results of a double-feeding experiment, plant a. with growing-point removed and plant b. with root tips removed. Although there is an obvious difference in distribution between the plants, two subsequent trials failed to verify this and it was concluded to be an artifact. Figure 2 is included merely to illustrate the sensitivity of the technique. Interesting detail may be seen in the activity of the root in Figure 2,a. The fact that no detectable activity was found in the primary leaves and cotyledons (designated p and c) was also noted by Vernon



a b

Figure 2. Autoradiograms of 20 min, C^{14}-photosynthate translocation

a. growing point removed, b. root tips removed.

(60). This and the discontinuities in stem, activity distribution were verified in later sectioning experiments.

Expt. 1,b. C^{14} distribution; 20 min, $C^{14}O_2$, leaf administration; steamed stem

Heat treatment of stem tissue may be used to destroy the living tissue without interfering with transpiration. Vernon's experiments (60) on isolating stem sections by ringing with hot wax indicated a predominant "source-sink" influence on translocation. It was decided to investigate the effect, on photosynthate movement, of steam-killing a short section of the stem.

Two very similar plants, grown in the same pot, were administered a total of about 200 microcuries of $C^{14}O_2$ by employing two leaf chambers in series with the reaction vessel, the treated plant being first in line. A 2 cm section, just below the cotyledon node of the first plant was previously killed by playing a fine jet of steam around its periphery, for about 20 minutes. This region was chosen because of its structural duribility. Although it took on a watery, cooked appearance, it would still support the stem whereas stem regions above the cotyledon would immediately collapse.

In two preliminary, 20 and 30 minute trials, no activity was found below the steamed section and rough activity measurements showed considerably reduced translocation in the stem compared to the controls. One further 20 minute

experiment was carried out and the plant was sectioned and analysed. The results are presented in Table 1 and the stem section activities, in Figure 3. The 3 cm, petiole section, protruding from the leaf chamber was also analysed. Its activity is included in the total stem data and in Figure 3, is multiplied by two-thirds to make it comparable with the activity in the 2 cm stem sections. The latter are numbered from the entrance petiole downward.

Note that the stem activity in the treated plant is considerably less than that in the control even though its leaf obviously received the greater share of $C^{14}O_2$. On the assumption that the leaf activity is a measure of the radioactive photosynthate available for transport, the relative amounts moved into the stems are 2.4 and 5.2 percent for the treated and control, respectively; into the growing points, 1.8 and 1.2 percent.

Expt. 1,c. C^{14} distribution; 15 - 30 min, C^{14}-photosynthate, petiole administration

Sisler, et al. (53) used the petiole feeding technique in tomatoes for comparing 2 - 7 hour translocations of C^{14}-sucrose, in boron-deficient and normal plants. The amount of activity incorporated by this method appeared to be comparable with that incorporated by leaf administration of $C^{14}O_2$. This would appear to excuse the leaf from playing any role in movement except that of being the source of supply. It

Table 1. C^{14} distributions; 20 min, $C^{14}O_2$, leaf administration; one plant with steamed stem, the other intact

Section	Total activity c/m[a]	
	Steamed	Intact
Leaf	76,300,000	59,600,000
Growing point	1,360,000	723,000
Primaries	510	846
Cotyledons	23	15
Stem	1,800,000	3,110,000
Petiole	737,000	973,000
1	379,000	1,000,000
2	473,000	559,000
3	306,000	352,000
4	113,000	251,000
5	7,900	104,000
6	589	53,400
7		23,800
8	20	8,640
9	61	2,420
10		490
11		83

[a]Three significant figures only.

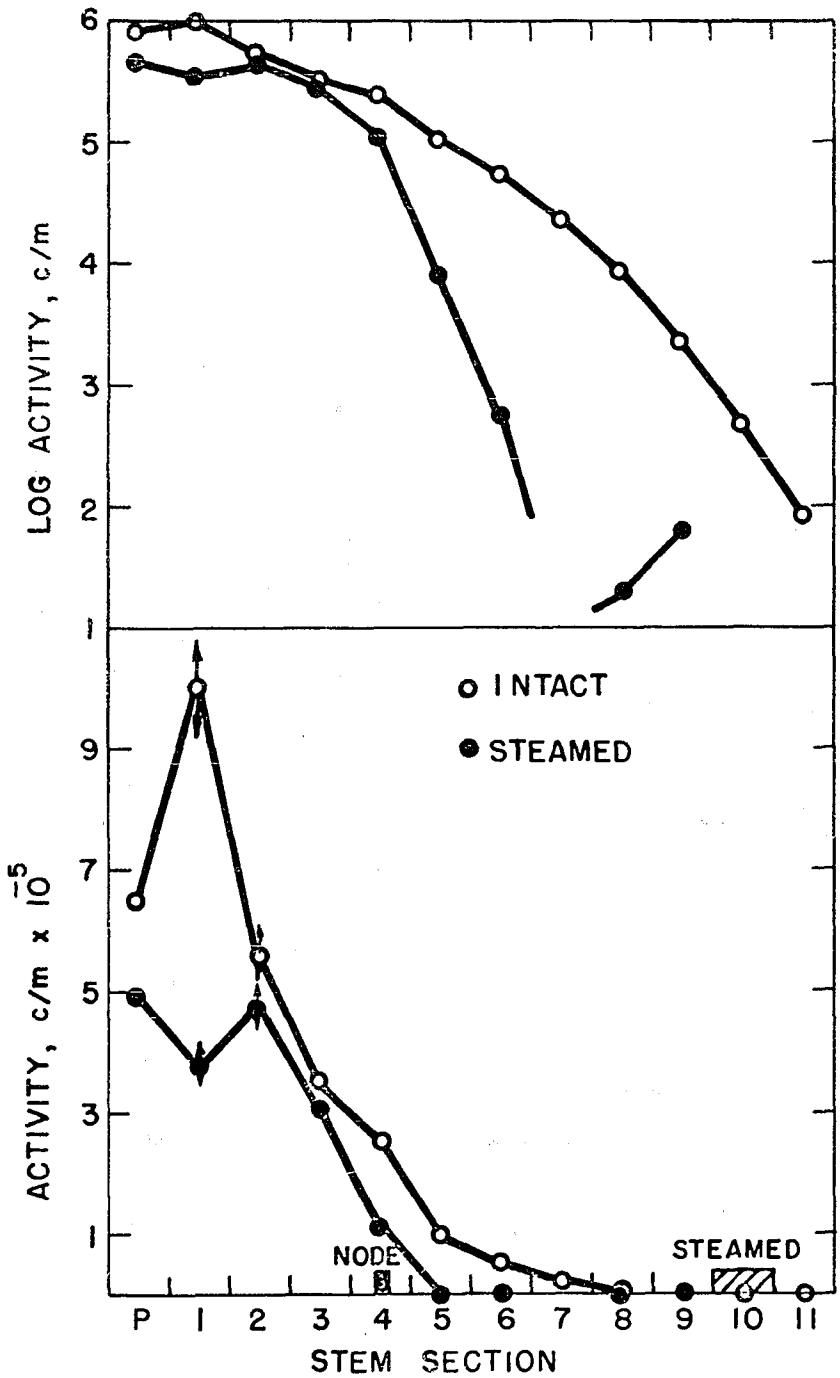


Figure 3. Total, stem-section activities following 20 min, $C^{14}O_2$, leaf administration; one plant steamed below the cotyledon node

seemed pertinent, therefore, to compare the distribution of C^{14}-photosynthate applied in this manner with that of the $C^{14}O_2$-fed plants in Expt. 1,b. Only a small quantity of uniformly labelled sucrose (specific activity, approximately 10^3 counts per minute per mg) was available. This was administered through the cut petiole of a plant as a 7 percent solution, and the growing point and entire stem were analysed for C^{14} activity after a 30 minutes translocation period.

A further trial was performed with uniformly labelled C^{14}-fructose (in larger supply) of specific activity, 2.3 x 10^6 counts per minute per mg, fed as a 1/2 percent solution. With this it was possible to measure the activity in 2 cm stem sections after a 15 minutes translocation period. Table 2 presents the section activity results. The stem distribution for the C^{14}-fructose experiment is shown in Figure 4.

Expt. 1,d. Effect of removing the root on C^{14} and Cl^{36} movement

Four double-plant experiments were performed in which leaves and petioles were administered tracer in standard amounts for 30 minutes; one plant being severed below the cotyledon node and the cut stem, maintained in tap water. The cut plants were treated at least one hour before the experiment and appeared turgid and normal. The tracers and method of administration were: 1) $C^{14}O_2$, leaf 2) HCl^{36} vapor, leaf 3) C^{14}-fructose, petiole 4) HCl^{36}, petiole.

Table 2. C^{14} distribution, petiole administration; 15 min, C^{14}-fructose, 9.3×10^6 c/m/ml; 30 min, C^{14}-sucrose, 6.2×10^5 c/m/ml

Section	Total activity c/m	
	C^{14}-fructose	C^{14}-sucrose
Growing point	4,620	559
Stem	44,100	5,130
1	6,180	
2	5,780	
3	6,500	
4	5,740	
5	9,040	
6	3,520	
7	2,650	
8	2,510	
9	2,180	

Simultaneous tracer administration was accomplished in 1) and 2) by placing two leaf chambers in series, as in Expt. 1,b. In 3) and 4) the two petioles were enclosed in the same feeding tube. The Cl^{36} administration and assay are detailed in Part II.

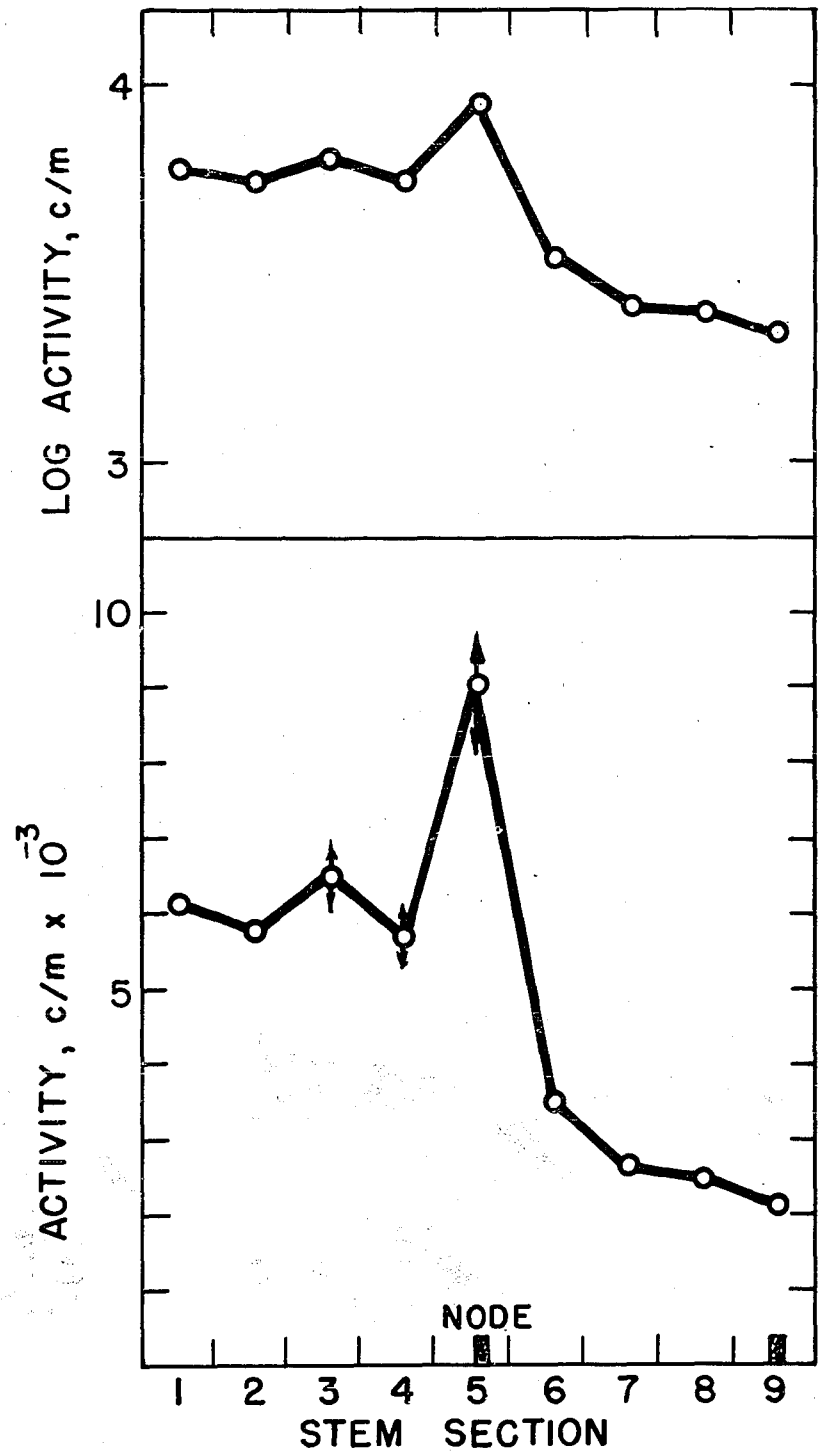


Figure 4. Total, stem-section activities following 15 min, C^{14}-fructose, petiole administration

The results were quite striking. While the control plants translocated tracer in the expected manner, the cut plants, in every case, were markedly inert. Small amounts of activity (from 1 to 10 percent of that in controls) were recovered in petioles and first adjacent stem sections. A minute amount was observed in the growing point of the plant fed HCl^{36} vapor but the other three showed no significant activity above background.

Discussion

One must conclude that meristematic tissue plays no major role in photosynthate translocation in these short-period trials. There is no apparent "pulling force" from these regions. The ease with which materials move into plants via cut petioles would also indicate that there is no "pushing force" from the leaf.

The distribution, amount and rate of movement appears to be roughly similar whether photosynthate is supplied by the leaf or through the cut petiole. Although a quantitative comparison is not justified at this point, later experiments confirm this view and a comparison is made between the two feeding techniques in Part II.

The discontinuities in the stem distribution curves in Figures 3 and 4 are of interest. Similar maxima and minima were observed in later experiments. They are believed to

be significant even though the experimental errors are unavoidably large with the techniques used. The measurement error was estimated to be about plus or minus 8 percent from consideration of uncertainties in length of section, extraction and counting. These limits are indicated by the vertical arrows on the linear plots. The semilog plots confirm the general exponential decay nature of activity as a function of stem distance, observed by others. It is interesting to note how the discontinuities are smoothed out by this plotting technique.

Steaming a lower region of the stem, as in Expt. 1,a, exerts an unquestionable inhibitory influence on downward movement. Though there is a small increase in activity after section 7 of the treated plant, there is no major accumulation at the steamed section. It is clear that the action of killing an isolated portion of the lower stem effects movement, from the source, into preceding sections.

The results of Expt. 1,d would appear to indicate that the root has a predominant influence on downward (and, indeed, upward) movement of photosynthate. The possibility exists, however, that the one hour pretreatment was insufficient time to permit shock recovery by the plants.

Barring the above mentioned possibility, the conclusions drawn from these experiments are that some nonaerial region of soybean plants (root tips excluded) has a controlling

influence on photosynthate movement. Steam killing of a lower stem region would appear to reduce this influence.

PART II. SIMULTANEOUS CHLORIDE MOVEMENT

Regardless of the actual mechanism for photosynthate translocation, it is of interest to inquire: 1) Is there a common vehicle for movement of photosynthate and other materials? 2) Is the translocate itself involved in some necessary metabolic complex? It would seem useful, therefore, to compare the movement of a nonmetabolite with that of photosynthate.

Swanson and Whitney (57) found an independent rate and distribution for Cs^{137} when applied with P^{32} in solution, to kidney bean leaves, during 1.5 - 4.5 hour translocations. It is known that this method of administration results in relatively small incorporation of tracer and it seemed possible that distributions obtained after these lengths of time would not be faithful representations of the moving flux.

Consequently, a search was made for a radioactive nonmetabolite that could be introduced to leaves, in gaseous form with $C^{14}O_2$. Cl^{36} was decided upon because it is known that chloride ion plays no major role in plant metabolism and this isotope has a long half-life with a strong beta emission.

Experimental

Cl^{36} administration and assay

Three week old soybeans, similar to those used in Part I, were fed Cl^{36} to trifoliates, in the form of HCl^{36} vapor, using the same apparatus as for the $C^{14}O_2$ administration in Expt. 1,b. Thirty - sixty microliters (1 - 2 microcuries) of the 1.64 N acid was added to the reaction vessel and this was released with an excess of anhydrous H_2SO_4. In dark-leaf administration, the plant and feeding apparatus were set up in a light-tight fume hood for about one hour prior to the release of the radioactive gas. Petioles were fed HCl^{36} solutions (1 - 2 microcuries per ml in about 0.05 N acid) using the same technique as for Expt. 1,c. Activity was extracted in an identical manner to that of the C^{14} samples in Part I.

The energetic emission of Cl^{36} permitted a rough measure of the time-course of translocation by placing a thin-window geiger tube adjacent to the plant tissue. In Expts. 2,a and b, extract activities were measured with the geiger tube. Because of the difference in beta energies (0.66 Mev for Cl^{36} and 0.15 Mev for C^{14}) it was possible, in simultaneous C^{14} - Cl^{36} experiments, to count the two activities, from the same extract, using a 0.07 mm aluminum filter. This was placed 3 mm above the sample and it was found to transmit 73 percent of the Cl^{36} betas and only 0.5 percent of the C^{14} betas.

This relationship held approximately over the range of activities used. For Expt. 2,c, the more sensitive windowless flow-counter was used. Here, the filter was held 1 cm above the sample and, with this geometry, it transmitted only 0.04 percent of the C^{14} betas. To aid in calculations, the calibration curves in Figure 5 were obtained from two accurate dilution series.

Chromatographic identification of Cl^{36} ion

A two dimensional, paper chromatographic procedure was developed using solvents suggested by Yamaguchi (63). The 80 percent ethanol, plant extracts were shaken with petroleum ether to remove pigments (this component contained negligible activity and was discarded) and the alcohol-soluble fraction was reduced to small volume, by vacuum evaporation, prior to chromatography. This was spotted on one corner of a square of Whatman No. 1 filter paper which was then stapled in the form of a cylinder and enclosed in an ascending chromatographic jar. [Aronoff (4)] . Good separation was affected by developing in one direction with 1 part ethyl acetate - 1 part ethanol - 1 part normal ammonium hydroxide and in the other direction with 3 parts acetone - 1 part water. After development, the radioactive spots were located by means of an automatic chromatogram scanner [Aronoff (3)] . Rf's were determined with nonradioactive chloride by spraying lightly

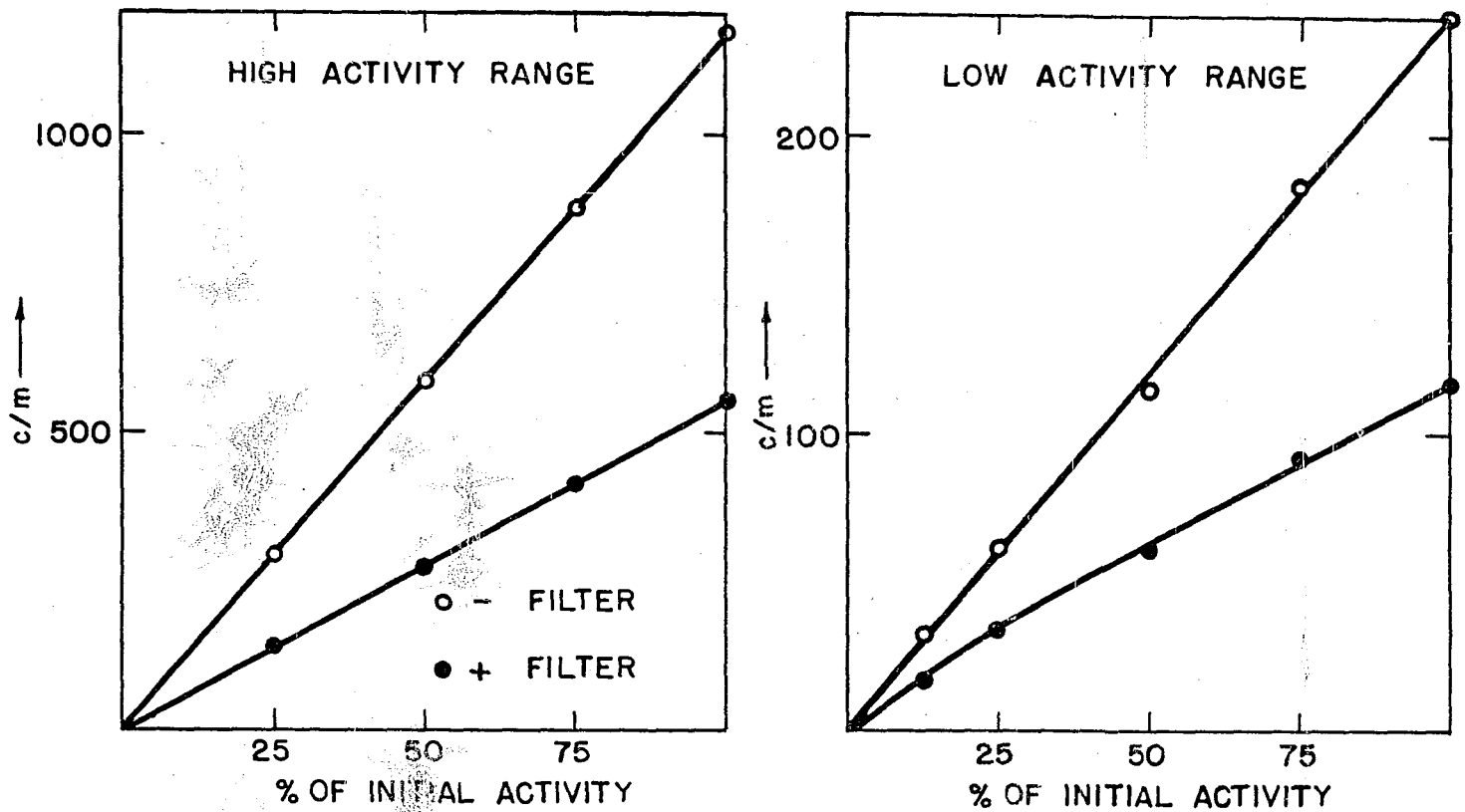


Figure 5. Cl^{36} calibration of the windowless flow-counter with a 0.07 mm, Al, C^{14}-beta filter placed 1 cm above the sample

with a dilute silver nitrate solution. The chloride spots darken quickly upon exposure to ultraviolet light.

Results

Expt. 2,a. Cl^{36} distribution; HCl^{36}, leaf administration; light and dark; identification of Cl^{36-} in plant extracts

Initial attempts to introduce Cl^{36} via illuminated leaves were unsuccessful. The trifoliates were quite susceptible to injury (they became curled and twisted) from the HCl^{36}, the tolerance level being about 0.1 percent by volume of the acid vapor. Finally it was found that measurable activity was translocated in plants that had been kept in subdued light for 12 - 24 hours; or that were administered tracer in the dark. In the latter case, the leaves and petioles could tolerate up to three times the concentration of acid vapor as the illuminated plants.

In the successful trials, an anomalous, ultra-rapid, downward movement of activity was observed with the geiger monitor. Significant activity would appear in the primary leaves within one minute after release of the tracer to the leaf. It is believed that this was not caused by leaks in the system because it was still observed when the reaction flask was first evacuated so as to produce an initial reduction of pressure of about 5 cm of water, in the gas

system; and the fume hood air flow was directed upward over the plant.

In an attempt to establish whether or not Cl^{36} moved in the free ionic form, three light, leaf feedings were performed. The stem activity was removed by grinding and extracting first with boiling 80 percent ethanol, second with boiling water and finally with cold water. The radiochromatograms, in all three cases gave well resolved Cl^- spots.

Two detailed distribution analyses were made; one of a leaf-fed plant in the light and the other of a leaf-fed plant in the dark. The results are given in Table 3 and in Figures 6 and 7.

Expt. 2,b. Simultaneous, leaf administration, HCl^{36} and $C^{14}O_2$

The obvious next step was to attempt simultaneous feeding of the two gaseous tracers to the same leaf of a plant. Two closed reaction flasks (Figure 1,c) were arranged in series with the leaf chamber and the tracers were liberated separately under different degrees of vacuum. The adjoining stopcock was then opened so that the two were mixed before release to the leaf chamber. Three plants that had been confined in subdued light for about 48 hours were given the following microcurie amounts of the two tracers, for 15 minutes: 1) 2 HCl^{36} - 2 $C^{14}O_2$. 2) 2 HCl^{36} - 125 $C^{14}O_2$. 3) 1 HCl^{36} - 150 $C^{14}O_2$. The plants were sectioned and the

Table 3. Cl^{36} distribution, leaf administration, HCl^{36}; 25 min, light, 1 microcurie; 15 min, dark, 2 microcuries

Section	Total activity c/m		dark c/m / light c/m
	Light	Dark	
Leaf	67,800	178,000	2.6
Growing point	697	2,780	4.0
Primaries	763	1,740	2.3
Stem	2,950	18,300	6.2
1	633	1,630	
2	464	1,990	
3	322	2,480	
4	372	2,140	
5	206	2,030	
6	230	1,950	
7	183	1,740	
8	192	1,510	
9	185	1,600	
10	164	940	

Figure 6. Total, stem-section activities following 25 min, HCl^{36}, leaf administration in the light

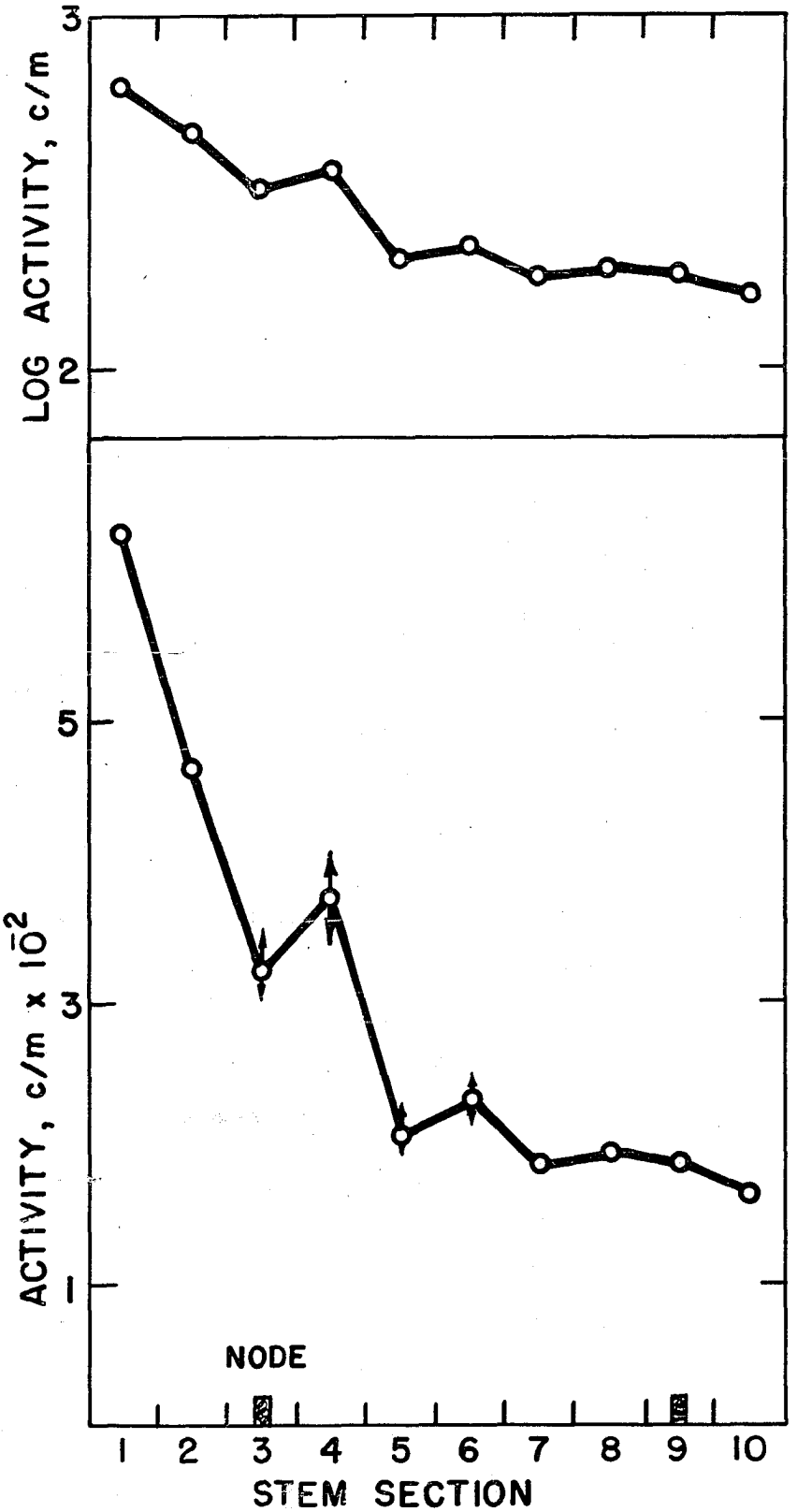
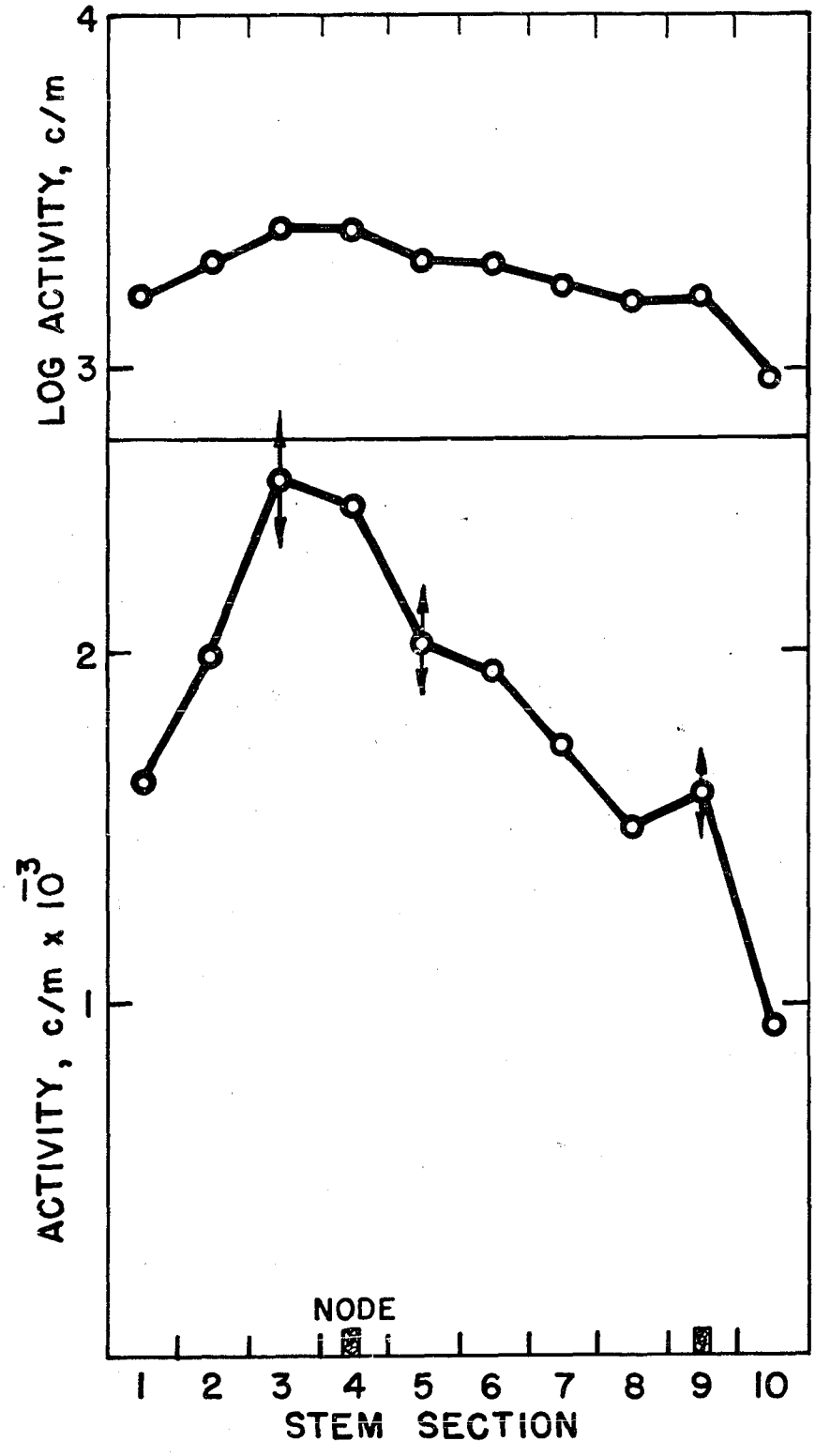

LOG ACTIVITY, c/m
3
2
ACTIVITY, c/m x 10⁻²
5
3
1
NODE
1 2 3 4 5 6 7 8 9 10
STEM SECTION

Figure 7. Total, stem-section activities following 15 min, HCl^{36}, leaf administration in the dark


LOG ACTIVITY, c/m
4
3
2
1
ACTIVITY, c/m x 10⁻³
NODE
1 2 3 4 5 6 7 8 9 10
STEM SECTION

extracts were assayed for the two tracers simultaneously. In each case, although expected amounts of Cl^{36} were recovered, the quantities of C^{14} obtained from stem sections were too small to measure and the experiment was abandoned. The data for the second trial together with a sample calculation are given in Table 4.

Expt. 2,c. Simultaneous, petiole administration, HCl^{36} and C^{14}-fructose

It seemed reasonable, as a final approach, to attempt simultaneous application of the tracers to a cut petiole. In a preliminary run, using HCl^{36} alone (1 microcurie per ml), appreciable activity was indicated by the monitor at the lower stem after 15 minutes. Next a cut petiole was fed a mixture containing about 1.5 microcuries, HCl^{36} per ml and 10^7 counts per minute per ml of the C^{14}-fructose used in Expt. 1,c. The detailed results are given in Table 5 and Figure 8. A 3.2 cm length of the petiole adjacent to the stem was also analysed. Its activity in Figure 8 is obtained by multiplying the total by 0.625 to make it comparable to the 2 cm stem sections.

Table 4. C^{14} and Cl^{36} distribution; 15 min, simultaneous leaf administration; 2 microcuries HCl^{36}, 125 microcuries $C^{14}O_2$

Section	Total activity c/m				
	- filter	+ filter	Cl^{36}	C^{14}	C^{14}/Cl^{36}
Leaf (x 10^5)	5.67	1.76	2.39[a]	3.28[a]	1.37
Growing point	1,270	800	1,100	174	0.16
Primaries	1,920	1,400	1,916		
Stem 1	1,890	1,360	1,870	20	0.02
2	2,180	1,620	2,200		

[a]Sample calculation

1. $Cl^{36} + C^{14} = 5.67 \times 10^5$
2. $0.73\ Cl^{36} + 0.005\ C^{14} = 1.76 \times 10^5$
2. (x 1.37) $Cl^{36} + 0.00685\ C^{14} = 2.41 \times 10^5$

$0.993\ C^{14} = 3.26 \times 10^5$

$C^{14} = \underline{3.28 \times 10^5}$

$Cl^{36} = (5.67 \times 10^5) - (3.28 \times 10^5)$

$Cl^{36} = \underline{2.39 \times 10^5}$

Table 5. Cl^{36} and C^{14} distribution; 15 min, simultaneous, petiole administration; HCl^{36}, 2.28 x 10^6 c/min/ml; C^{14}-fructose, 5.75 x 10^6 c/min/ml

Section	Total activity c/m		Cl^{36}/C^{14} %
	Cl^{36}	C^{14}	
Fed solution			39.6
Growing point	1,040	4,520	23.0
Stem	10,710	33,540	32.0
Petiole	3,700	9,850	37.6
1	1,380	3,780	36.5
2	1,200	2,760	43.5
3	1,350	2,890	46.7
4	1,120	4,110	27.2
5	890	3,840	23.2
6	440	2,500	17.6
7	360	1,980	18.2
8	170	1,190	14.3
9	100	640	15.6

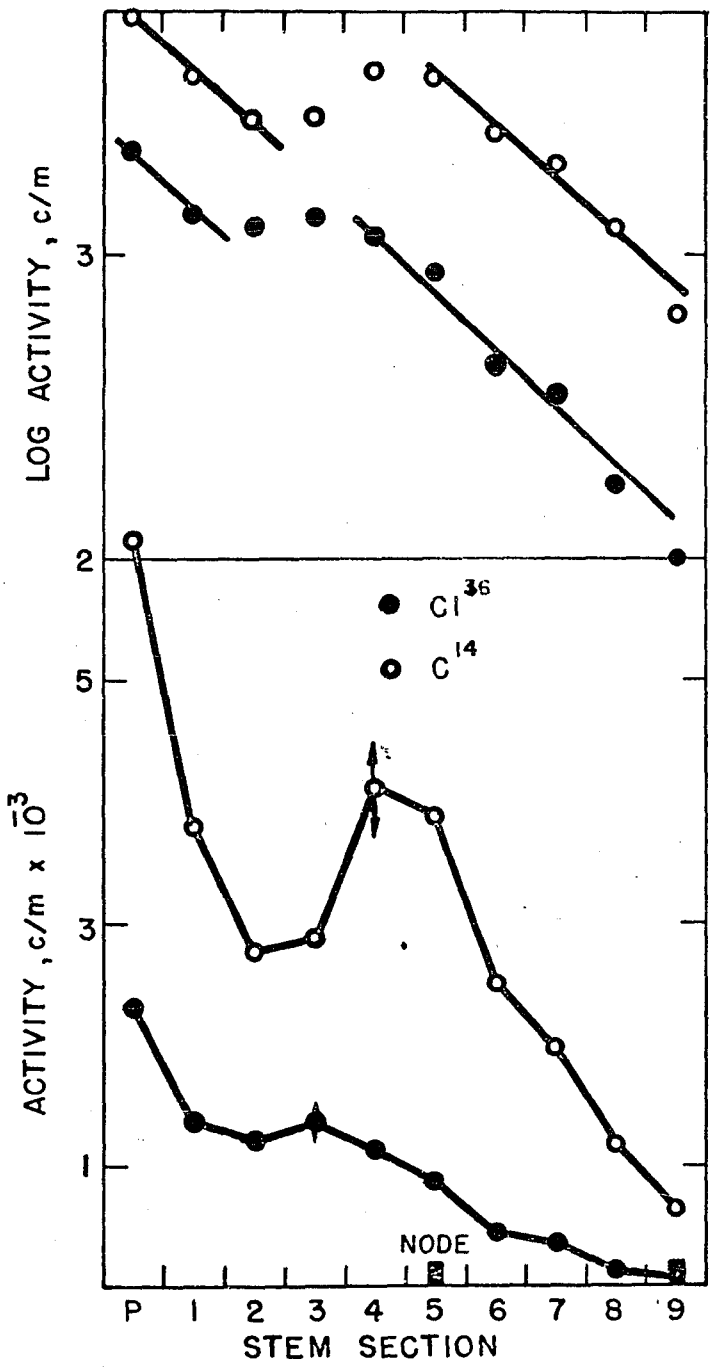


Figure 8. Total, stem-section activities following 15 min, simultaneous, petiole administration of HCl^{36} and C^{14}-fructose

Discussion

The following obvious conclusions may be drawn from these experiments:

a) Chloride moves readily from the leaf (but independently from photosynthate) under conditions of low photosynthate content of the leaf or from the darkened leaf (Expts. 2,a and b).

b) As far as could be determined, with the identification procedure used, it moves in the free ionic form (Expt. 2,a).

c) Although the stem distributions of Cl^{36} (Figures 6, 7, 8) appear similar in shape to those for C^{14}-photosynthate, there was considerable transport into the older, primary leaves. The amounts in the primaries relative to amounts in the fed leaves of Expt. 2,a (Table 3) were two to three orders of magnitude greater than the same recovery ratio for C^{14} in Expt. 1,b. This is in qualitative agreement with the observations of Swanson and Whitney (57) who noted different rates of movement for three different tracers into leaves of kidney bean ($P^{32} > Cs^{137} > K^{42}$).

d) The very rapid appearance of Cl^{36} in the primary leaves, observed in Expt. 2,a, indicates an initial velocity of at least 7 m per hour. This same order of magnitude was observed by Nelson, *et al.* (50) for a small component of C^{14} activity following $C^{14}O_2$, leaf administration. This is, therefore, further evidence that there may be two separate

phases to the translocation process: a very rapid, small component overridden by the main phase. But, there is no way of knowing, from these experiments, that the rapid phase is maintained in the steady state.

It is clear that leaf, vapor feeding of HCl^{36} is not an appropriate technique for examining simultaneous movements of photosynthate and Cl^{36}. The almost complete lack of photosynthate movement in Expt. 2,b is probably related to interruption of photosynthesis by excess concentrations of hydrogen ion. The amount of C^{14} incorporated in the leaf relative to the amount applied (Table 4) was about a thousand times less than the same ratio for the singly applied $C^{14}O_2$ in Expt. 1,b. The distortion injuries observed in the early trials suggest tissue-turgor loss due to disruption of cell membranes (possibly by precipitation of associated protein complexes, caused by reduction of pH). However, the increase in HCl^{36} tolerance in the dark has photochemical implications. Likewise, the lack of chloride movement from the non-starved leaves in Expt. 2,a suggests inhibition of the transport mechanism, by some photochemical reaction depending upon the presence of photosynthate. This apparently was at work even in the starved plants of Expt. 2,b as may be seen from Table 3. Although the illuminated plant was administered twice as much tracer as the dark plant, the amounts incorporated and transported were lower by factors of 0.38 and 0.16 respectively.

That there is any relationship between these several factors, is not intuitively evident.

Table 6 is an attempt to summarize all relevant information regarding the two types of administration (leaf, vapor versus petiole, solution) and the two translocates (photosynthate versus chloride). The leaf and stem activities are expressed on a ml^{-1} basis assuming average values of 0.5 and 1 ml respectively for the plant juice.[1] The leaf, photosynthate values are expressed as sucrose activity assuming the 62 percent, incorporation value found by Vernon (60) for 20 minute feedings. It was also assumed, in all cases, that the source concentration remained invariant and that the amounts transported were directly time dependent. Accordingly, the stem activities were normalized for a 15 minute translocation period. It is felt that the similarity in molar ratios, within each feeding method, supports the notion that, during these short periods, chloride and photosynthate moved into the stem at the same rate.

The distinct reduction in this ratio for the petiole experiments is not unexpected, whether the solutes move actively or passively into the stem conducting channels. In either case, the action of severing the petiole undoubtedly reduces the entrance cross section. Indeed, it is difficult

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[1]Actual average values, obtained from five separate, fresh minus dry, mass measurements were 0.47 ml for the leaf and 1.1 ml per 20 cm of stem (3 percent standard deviation).

Table 6. Comparison of leaf-vapor versus petiole-solution administration methods for photosynthate and Cl^-

Expt.	Activity c/m/ml		% Molar ratio
	Source	Stem	
Leaf			
1b, $C^{14}O_2$, 20m Tbl 1, intact	7.40×10^7	2.33×10^6	3.1
3c, THO, 15m Tbl 9	5.20×10^5	2.08×10^4	4.0
2a, HCl^{36}, 25m Tbl 3, light	1.36×10^5	1.77×10^3	1.3
2a, HCl^{36}, 15m Tbl 3, dark	3.54×10^5	1.83×10^4	5.2
Petiole			
1c, C^{14}-f, 15m Tbl 2	9.3×10^6	4.41×10^4	0.47
1c, C^{14}-s, 30m Tbl 2	6.2×10^5	2.57×10^3	0.42
3b, C^{14}-f, 15m Tbl 7	1.4×10^7	5.17×10^4	0.37
2c, C^{14}-f, 15m Tbl 5	5.75×10^6	3.35×10^4	0.58
2c, HCl^{36}, 15m Tbl 5	2.28×10^6	1.07×10^4	0.47

to rationalize this difference in terms of any translocation concept which depends on active participation of cell membranes at the source, because absence of the leaf vascular network reduces any such "action surfaces" by many orders of magnitude.

The distributions of C^{14} and Cl^{36} in the simultaneous petiole feeding of Expt. 2,c are of interest (Table 5 and Figure 8). The orderly decrease in the stem Cl^{36}/C^{14} ratio and the noncoincidence of the discontinuity maxima might lead to the conclusion that different transport mechanisms are involved. On the other hand, the similarity in the semi-logarithmic slopes has intriguing theoretical inference.

Horwitz's analysis (31) for the comparable situation (see Appendix) depicts a hypothetical pipe, within the stem, being supplied a steady amount of radioactive material from a restricted external region. At any time, the slope of the plot of the logarithm of stem section activity versus stem distance is determined by three parameters related to the pipe: k, the diffusion constant or first order rate constant for removal of activity from the flowing stream into bound form; A_p, the cross sectional area and v, the velocity of fluid flow. Therefore, from the information available in Expt. 2,c only, there is no theoretical justification for assigning similar transport modes to the two tracers.

On the <u>a priori</u> assumption, however, that the same tissue is involved and that both tracers move at the same rate

(concluded from the analysis in Table 5), one is led to predict that the Cl^{36} and C^{14}-photosynthate, in this experiment, were transported in the same manner.

In order to support this hypothesis, two additional factors must be accounted for. In the first place, the model requires the same k for removal of the two components across the "pipe membrane". Horwitz (31) used the published value for sucrose of $2mm^2$ per hour (aqueous solution into water) for his model and obtained curves that were very similar to those found by Biddulph and Cory (6) with P^{32}. On this basis, it seems possible that a common vehicle is involved. Secondly, the displacement of the discontinuity peaks must be explained. It is the opinion of the author that this may be done, using the Horwitz model, by assumping the existence of a second source, S_2, (see Appendix) situated at stem section number 2 or 3 which would add material to the fluid flow in a similar fashion to that of the first (S_1). During the 15 minute period, this would contribute the bulk of the activity to the lower stem but would have a greater preference for supplying the C^{14} activity (hence, the shift in the C^{14} maximum). In the petiole, there is of course, only one external source at the immersed end. One must imagine, then, that S_2 is supplied, with internal connection, by S_1.

PART III. SIMULTANEOUS WATER MOVEMENT

At the time this investigation was started, there had been no reports of attempts to measure specifically the water content of the translocation flux. This seemed to be a pertinent aspect because, regardless of the transport system at work, it seemed reasonable to assume that an aqueous phase was involved. When tritiated water became readily available, it seemed possible to affect a separate measure of "solute" and "solvent". It was felt that such measurements might help resolve the questions that had been raised concerning the active or passive participation of solvent.

Experimental

THO administration and assay

THO was acquired initially in a concentration of 100 millicuries per ml. For safe handling, this was diluted to 10 millicuries per ml which, consequently, was the highest activity employed. The soybean plants, except where specified, were similar in age and development to those used in Parts I and II. In an attempt to eliminate any spurious results caused by internal water tensions, the plants were watered 10 to 15 minutes prior to feeding so that the pots were completely flooded.

Plants were either petiole-fed by immersing the petiole in THO solution, as in Part I, or were leaf-fed THO vapor. For the latter, the special bubbling apparatus, shown in Figure 9,a (containing 1 ml of 10 millicuries per ml, THO) replaced the reaction flask in the feeding apparatus used in Part I. The entire assembly was enclosed in a completely darkened fume hood and the vapor-saturated air was cycled over the leaf for one hour in the dark. Then a small quantity of pure CO_2 (to give about 0.1 percent by volume) was introduced to the gas system with a hypodermic syringe via the interconnecting, Tygon tubing. After a few minutes, the leaf was given about 1,000 ft-c of illumination, as in Part I. Zero experimental time was taken as that time when the lamp circuit was closed.

At the end of the feeding period, the plants were quickly sectioned and frozen, as in Part I, and water was removed from the sections in the following sequence:

a) The tissue was quickly transferred to one arm of the distilling apparatus shown in Figure 9,e, this arm being immersed in a Dewar flask containing liquid nitrogen.

b) The top valve was opened and the system was evacuated.

c) The top valve was closed and the Dewar, transferred to the other arm. The tissue arm was then surrounded by a steam bath and distillation, allowed to proceed for one hour.

d) The arm containing the distillate was quickly capped.

The sample was allowed to reach room temperature and was then assayed for THO activity.

THO assay was carried out by a modification of the NH_4Cl, exchange method described by Jenkins (34) as follows:

a) 200 mg per ml of NH_4Cl was dissolved in the THO-H_2O sample and was allowed to equilibrate for 10 minutes.

b) 0.1 ml of this solution was placed on an aluminum planchet and evaporated to dryness, at steam temperature, in the small bell jar illustrated in Figure 9,b. The pressure was regulated to prevent spattering until most of the water was removed and then full aspirator vacuum was applied for 10 minutes.

c) The assembly was removed from the steam bath, the vacuum seal broken and a Pyrex tube cylinder with a clean planchet was placed over the sample, as shown in Figure 9,c. The pressure was reduced to about 10^{-3} mm, Hg for 10 minutes.

d) The high vacuum was maintained and a hot plate, previously heated to about 200° C, was moved under the steel plate. The NTH_3Cl then sublimed slowly, condensing first on the inside of the cool glass cylinder, and finally, as the cylinder heated from the bottom, on the upper planchet. This gave an infinitely thick counting sample uniformly deposited over an area equal to the internal cross section of the cylinder; and insured uniform mixing in order to avoid any isotope effect.

e) The final sample planchet was allowed to cool to room

temperature and was counted in the windowless flow counter used in Part I.

This procedure differed from that described by Jenkins (34) only in the method of plating. He collected the dried, equilibrated sample, ground it to powder and spread it mechanically, in a uniform layer in a glass planchet.

Samples prepared in this manner, indicated progressively, decreasing disintegration rates (70 - 80 percent of the initial rate after about 10 minutes). If the sample plate was removed and allowed to stand in moving air for about half an hour or, if the counting gas was allowed to flow over the sample for the same length of time, with the anode potential reduced to zero; the same initial counting rate was observed followed, again, by the progressive decrease.

This hysteresis effect was also noted by Jenkins (34) and he recommended allowing sufficient time for the indicated disintegration rate to reach a steady value. It was found, however, that the same, steady-state value was not reproducible and several attempts were made to overcome the problem.

It seemed reasonable that this was a space-charge effect, the ionized $_2He^3$, disintegration product having insufficient recoil energy to escape the electrostatic field of the anode. Within the counter, then, these ions would accumulate in a layer just above the sample and some of the tritium betas would be captured before they could produce the necessary geiger avalanche. Attempts were made to install fine-mesh

grids of different geometries in the plate well just above the sample but, in all cases they reduced the detector sensitivity below tolerable levels. Samples plated on a thin copper disk showed no hysteresis effect when good electrical contact was maintained between the disk and plate holder. It was finally found that the difficulty could be overcome entirely, using aluminum planchets, by simply insuring good contact between the bottom of the plate and the earthed, plate holder, as illustrated in Figure 9,d. Subsequently, reproducible counts per minute could be obtained from the same sample planchet, from one day to another.

The entire assay procedure was standardized so that successive sample planchets, made from the same THO-H_2O sample, gave counts, above background, which varied less than plus or minus 3 percent from the mean.

That the disintegration rates obtained by this procedure were proportional to the molar concentrations of the THO-H_2O samples, is shown by the calibration curve in Figure 10. The points were obtained from a dilution series starting with the 10 millicuries per ml, THO (the uppermost point, by an initial dilution of 1,000). It should be noted that this does not give counts per minute as a function of absolute THO concentration. However, this was not considered to be important since the experimental conclusions were based on the measured activity, recovered in plant tissue, relative to

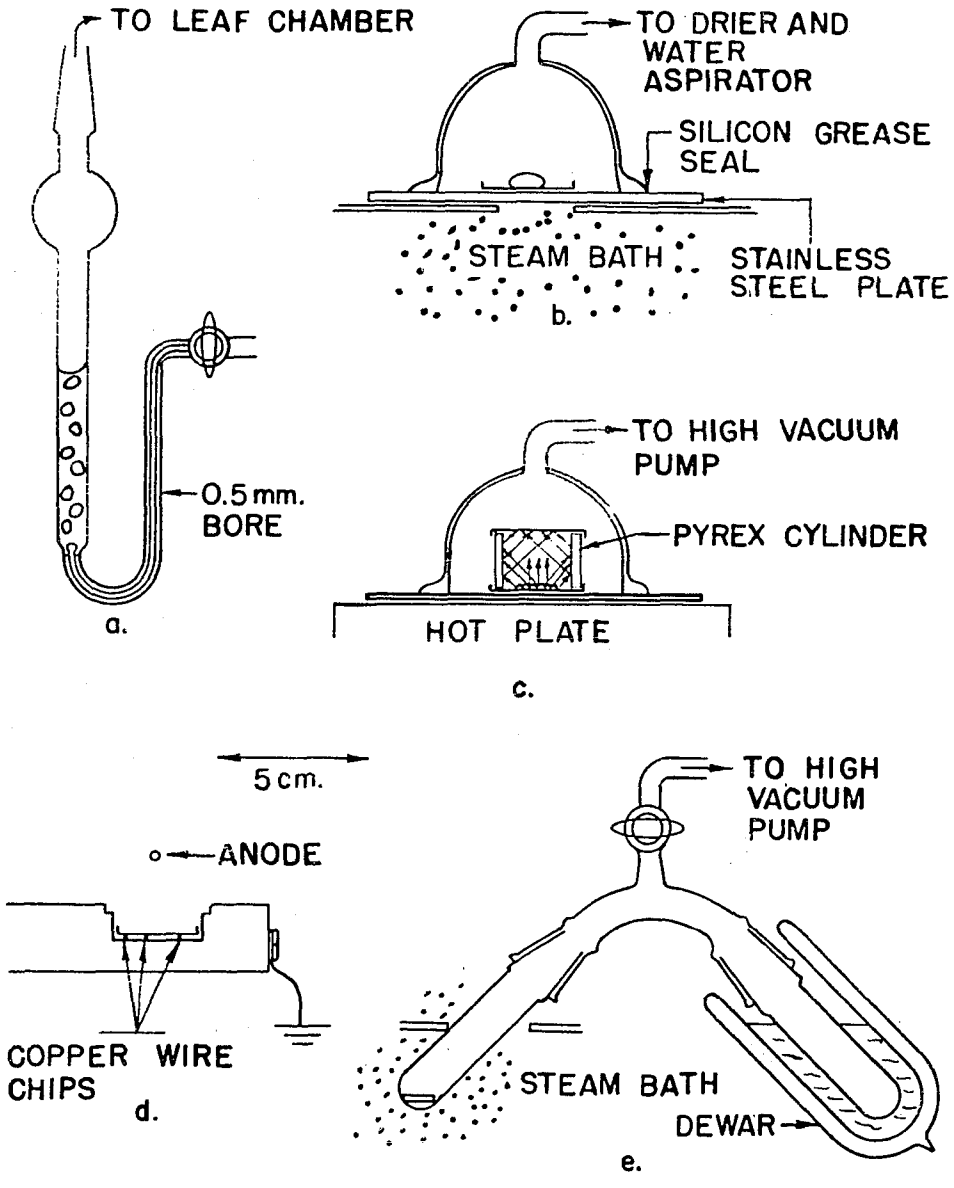


Figure 9. Apparatus for administration and analysis of THO

a. THO-vapor generator, b. and c. planchet, sample preparation, d. counter modification.

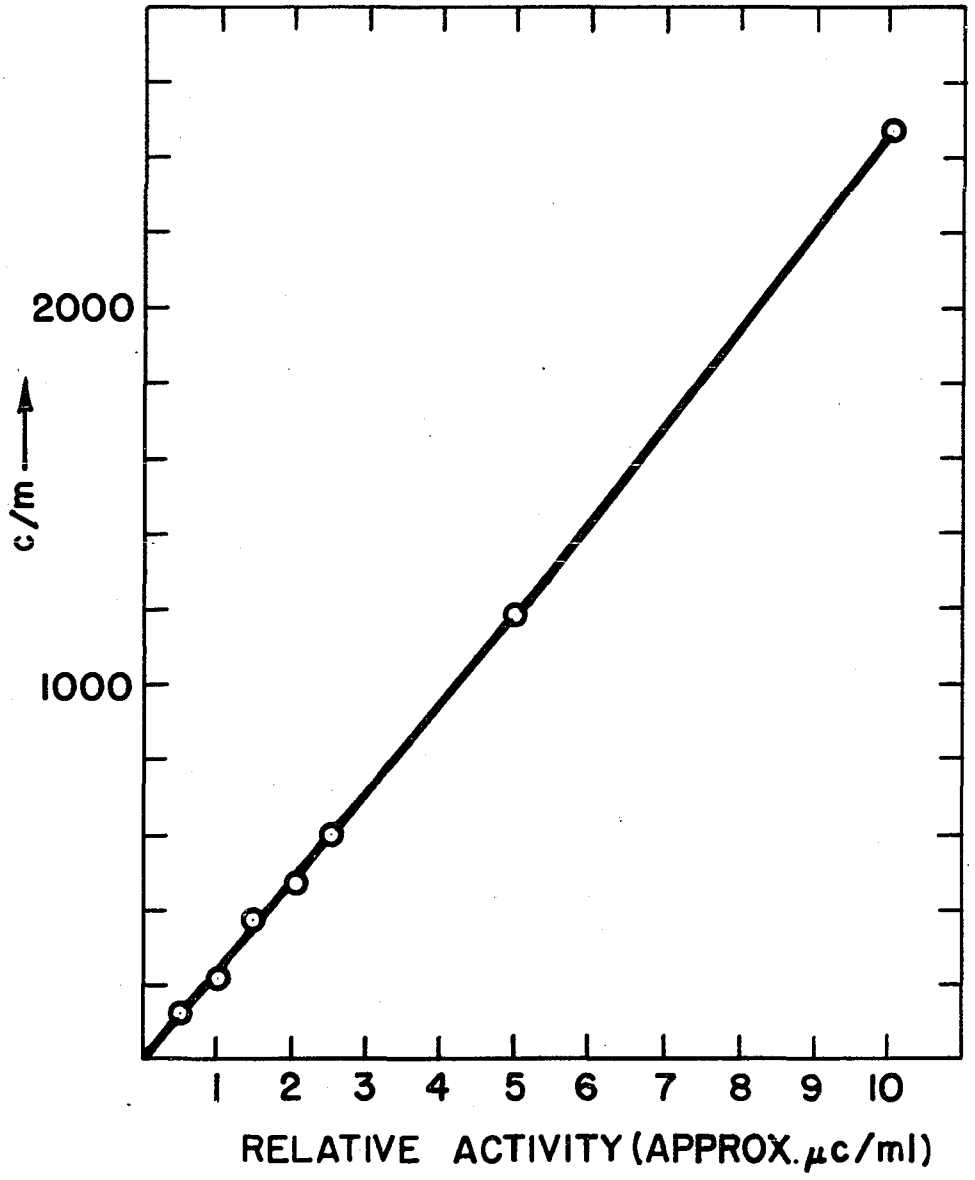


Figure 10. Calibration of NTH_3Cl method for THO analysis.

the measured activity introduced. This depends only on the linearity of the calibration curve.

T-photosynthate assay

The photosynthetically incorporated tritium was measured following the THO analysis. The dried sections were extracted with 80 percent ethanol and the extracts were sampled in a manner similar to the C^{14}-photosynthate extracts in Part I. The dried, planchet samples ranged in cross section from about 0.1 to 0.4 mg per cm^2 and a correction for self-absorption was necessary. The tritium, self-absorption curve of Figure 11 was constructed by the method indicated and this proved to be reasonably valid over the above range of cross sections. This correction, however, required a counting sample, mass measurement of from 1 - 2 mg and it was estimated that this increased the activity measurement error to about plus or minus 10 percent.

Results

Expt. 3,a. THO, petiole administration

From experience gained in Parts I and II, it was estimated, assuming an aqueous phase associated with the translocate, that detectable amounts of THO should be recovered from plant sections. For example, in Expt. 1,c, the C^{14}-fructose molar ratio between the upper stem sections and the

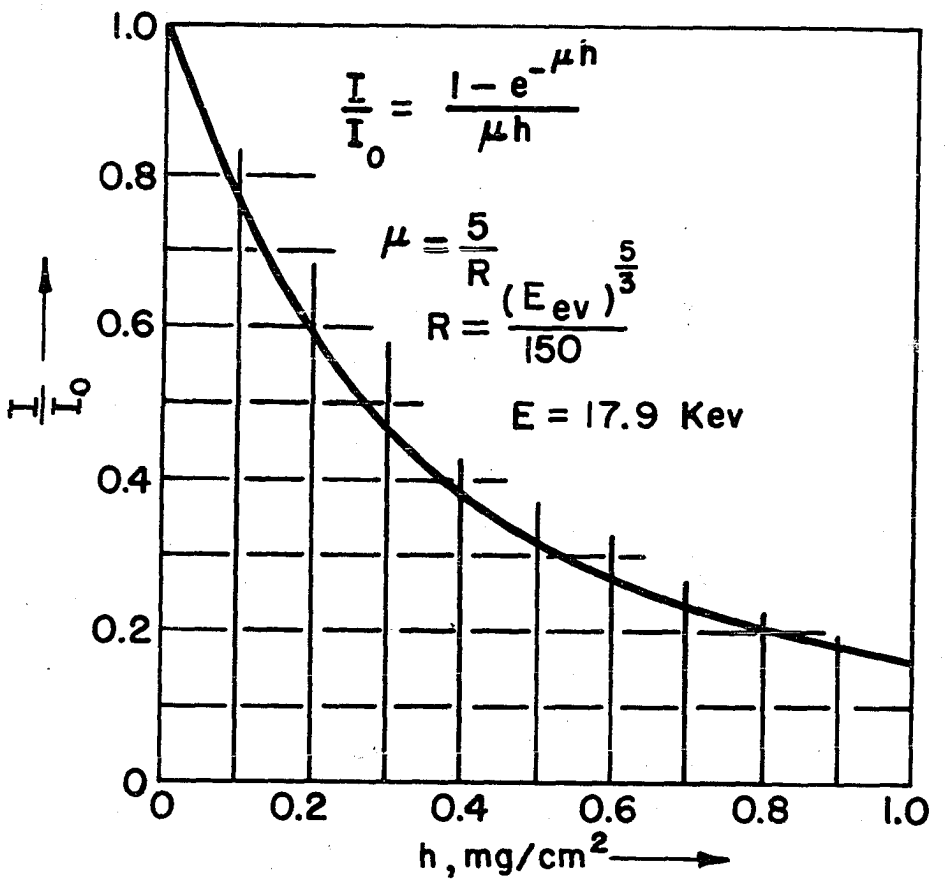


Figure 11. Self-absorption curve of tritium beta rays

The solid line was calculated from the upper expression where I and I_o are the indicated and actual activities; h is the sample cross section and μ is the absorption coefficient in cm^2/mg. μ was obtained empirically from the expressions involving the maximum beta range, R, in mg/cm^2 and the maximum beta energy, E, in Kev.

fed solution was about 0.6 percent. Assuming the same recovery ratio for THO, 50 microcuries per ml of fed solution (about 12,000 counts per minute, according to Figure 10) should yield about 70 counts per minute in the upper stem.

Two, preliminary 15 minute, petiole-feeding trials were performed with soybeans using 50 microcuries per ml, THO. In both cases, no detectable THO could be found in any of the plant sections.

Expt. 3,b. Simultaneous petiole administration, THO and C^{14}-fructose

Two experiments were carried out. In the first, a three-week-old soybean was petiole-fed a mixture containing the C^{14}-fructose used in Expt. 1,c and about 50 microcuries per ml, THO; for a feeding period of 15 minutes. Sections were assayed for THO and C^{14}, and the results are presented in Table 7. The C^{14}, stem distribution curve is shown in Figure 12.

As a check on the analysis procedure, the following was done: 1) 30 microliters of 10 microcuries per ml, THO were added to the distillate from an entire stem section (about 1 ml). This gave the expected THO assay. 2) 30 microliters of 1 and 10 microcuries per ml, THO were added, respectively, to two, fresh, 2 cm, stem sections (these contained about 0.1 ml of water). They were then sealed in small vials and, after two hours, frozen and analysed for THO. The distillates

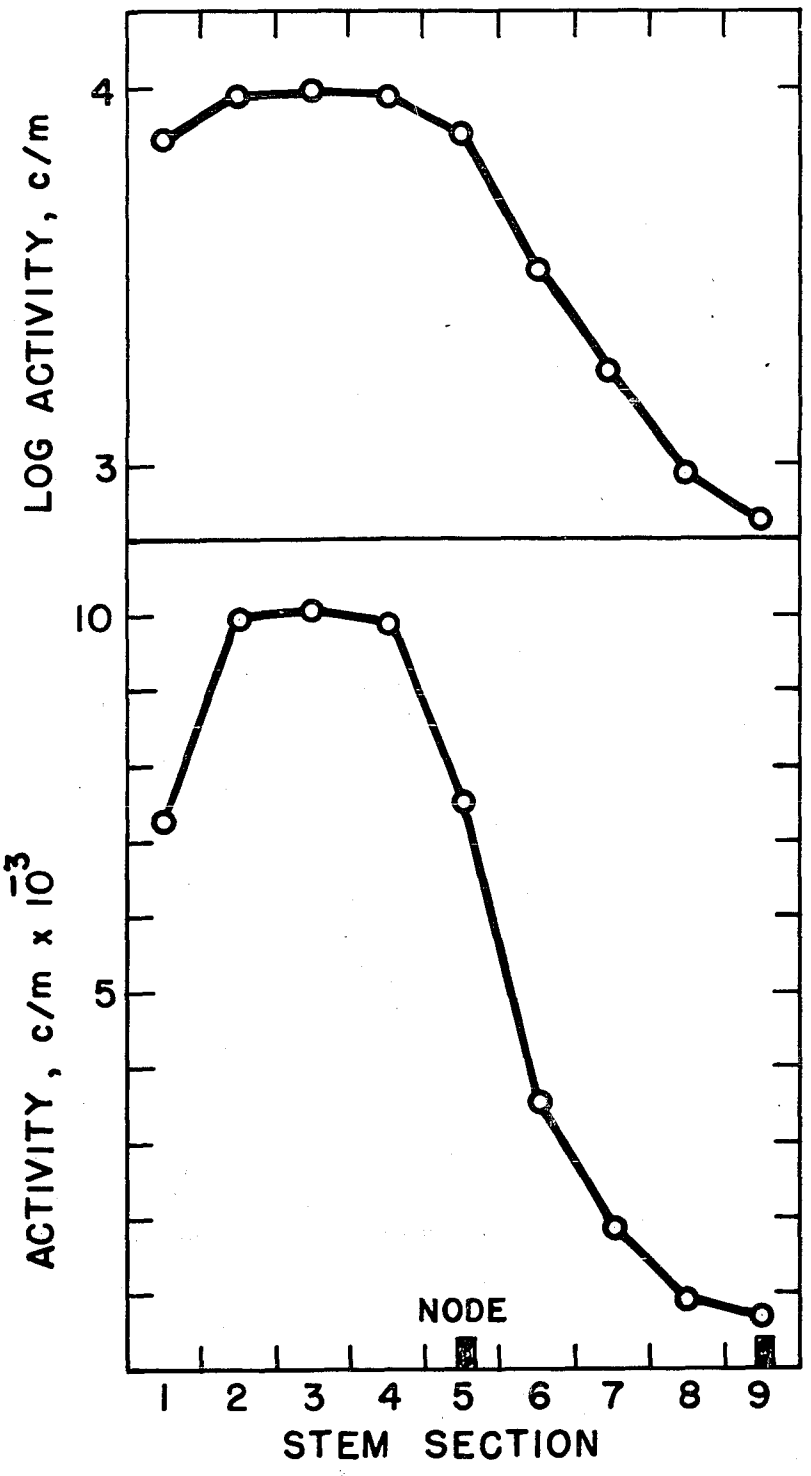
Table 7. THO and C^{14}-fructose distribution; 15 min, simultaneous, petiole administration; THO, 1.2×10^4 c/m/ml; C^{14}-fructose, 1.4×10^7 c/m/ml

Section	V[a] (ml)	C^{14}-f. (c/m)	THO (c/m/ml)	% Molar ratio[b] (section/source)	
				C^{14}-f.	THO
Gr. pt.	0.2	5,080	21	0.2	0.2
Stem		51,700			
1	0.1	7,220	12	0.5	0.1
2		9,950			
3		10,100			
4		9,860			
5		7,500			
6		3,560			
7		1,830			
8		950			
9		730			

[a]Volume of the extracted water, accurate to ± 0.02 ml.

[b]Calculated assuming that V is a measure of the tissue volume.

Figure 12. Total, stem-section, C^{14} activity following 15 min, simultaneous, petiole administration of THO and C^{14}-fructose


LOG ACTIVITY, c/m
4
3
ACTIVITY, c/m x 10^-3
10
5
NODE
1 2 3 4 5 6 7 8 9
STEM SECTION

gave counts per minute, above background, of 42 and 394, respectively.

As a check on the possibility of dilution of any transported THO by transpiration, the second trial was done with a five-week-old soybean with two, fully-developed trifoliates spaced about 5 cm apart on the stem. The experiment was performed in still air at ordinary room temperature and illumination. The oldest, trifoliate petiole was fed a C^{14}-fructose, THO mixture as in the first experiment except that the THO concentration was increased to 5 millicuries per ml and the experimental time was reduced to 6 minutes. All of the plant parts were assayed for THO and C^{14} activity except the newest trifoliate (which was lost) and the stem sections below number 9. The fed petiole was divided into two, 6 cm lengths. These were designated P_1 and P_2. P_1 was dipped into the radioactive solution and was rinsed quickly with water prior to freezing. The experimental results are given in Table 8 and the C^{14} stem distribution, in Figure 13. Activity was found only in the stem and the rest of the plant parts were not included in the results. The P_1 and P_2 activities in Figure 13 were normalized to a 2 cm length by dividing the total activities by a factor of three. For convenience in plotting, they were placed between stem sections 3 and 4 and are not comparable, in their abscissa displacements, to the stem-section, activity points.

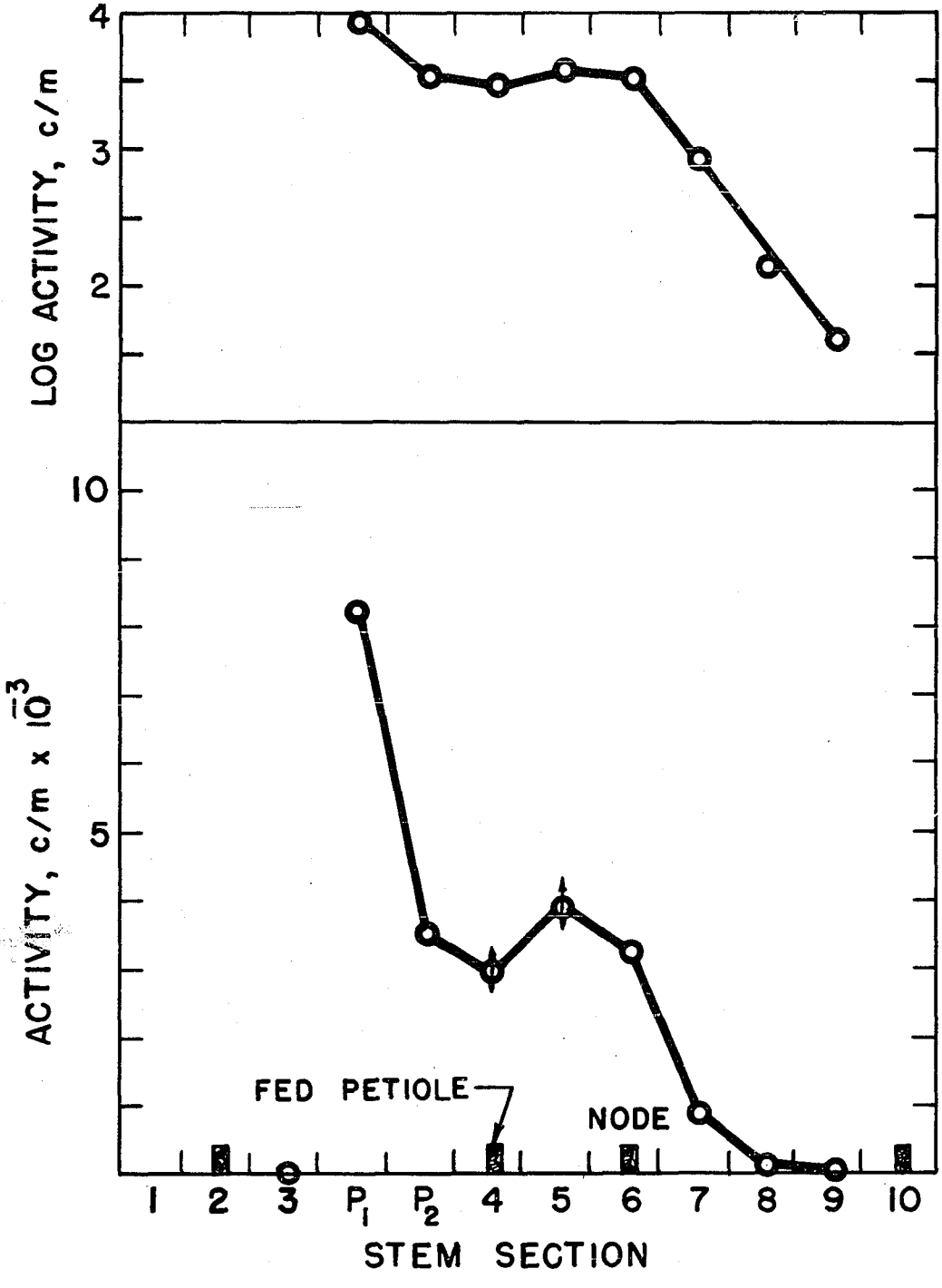
Table 8. THO and C^{14}-fructose distribution; 6 min, simultaneous, petiole administration; THO, 1.2×10^6 c/m/ml; C^{14}-fructose, 1.4×10^7 c/m/ml

Section		V[a] (ml)	C^{14}-f. (c/m)	THO (c/m/ml)	% Molar ratio[b] (section/source)	
					C^{14}-f.	THO
	P_1	0.07	24,600	8,240	2.5	0.7
	P_2	0.07	10,600	79	1	0.007
Stem	1					
	2					
	3		120			
	4	0.1	2,960	3	0.2	0.0003
	5		3,870			
	6		3,240			
	7		830			
	8		140			
	9		40			
	10	(not analysed)				

[a]Volume of extracted water, accurate to ± 0.02 ml.

[b]Calculated assuming that V is a measure of the tissue volume.

Figure 13. Total, stem-section (and normalized petiole-section), C^{14} activities following 6 min, simultaneous, petiole administration of THO and C^{14}-fructose


LOG ACTIVITY, c/m
4
3
2
ACTIVITY, c/m x 10⁻³
10
5
FED PETIOLE
NODE
1 2 3 P1 P2 4 5 6 7 8 9 10
STEM SECTION

Expt. 3,c. THO-vapor, leaf administration

As a final attempt, it was decided to analyse the translocate from leaves that had been equilibrated in the dark with THO-vapor prior to a photosynthesis period. The administration and assay procedures are detailed under Part III, Experimental.

In the first experiment, a three-week-old soybean was analysed following a 15 minute light period. The results are presented in Table 9 and the T-photosynthate, stem-section activities, in Figure 14.

An 18-day-old cucumber, Cucurbita sativus L. (variety unknown), was used in Part IV for autoradiography following a 30 minute light period. The remaining sections were also analysed for T-photosynthate and THO. The T-photosynthate, stem-section activities, together with the method for designating the plant parts, are shown in Figure 15. The detailed analysis is given in Table 10. THO activities below 8 counts per minute above background are indicated as "trace".

Table 9. Soybean, THO and T-photosynthate distribution; 15 min light period following 1 hr equilibration in the dark with saturated vapor from 10 mc/ml, THO

Section	V[a] (ml)	T-ph. (c/m)	THO (c/m/ml)	% Molar ratio[b] (section/source)	
				T-ph.	THO
Tri.	0.82	689,000	398,000		
Gr. pt.	0.25	3,500	175	2.7	0.04
Stem		20,800			
1	0.1	3,950	19	7.6	0.005
2	0.1	2,950	7	5.7	0.002
3		5,420			
4		3,670			
5		2,070			
6		1,510			
7		960			
8		249			

[a]Volume of extracted water; stem sections accurate to ± 0.02 ml.

[b]Calculated assuming that V is a measure of the tissue volume. The trifoliate activity was normalized to 62 percent sucrose, as in Part II.

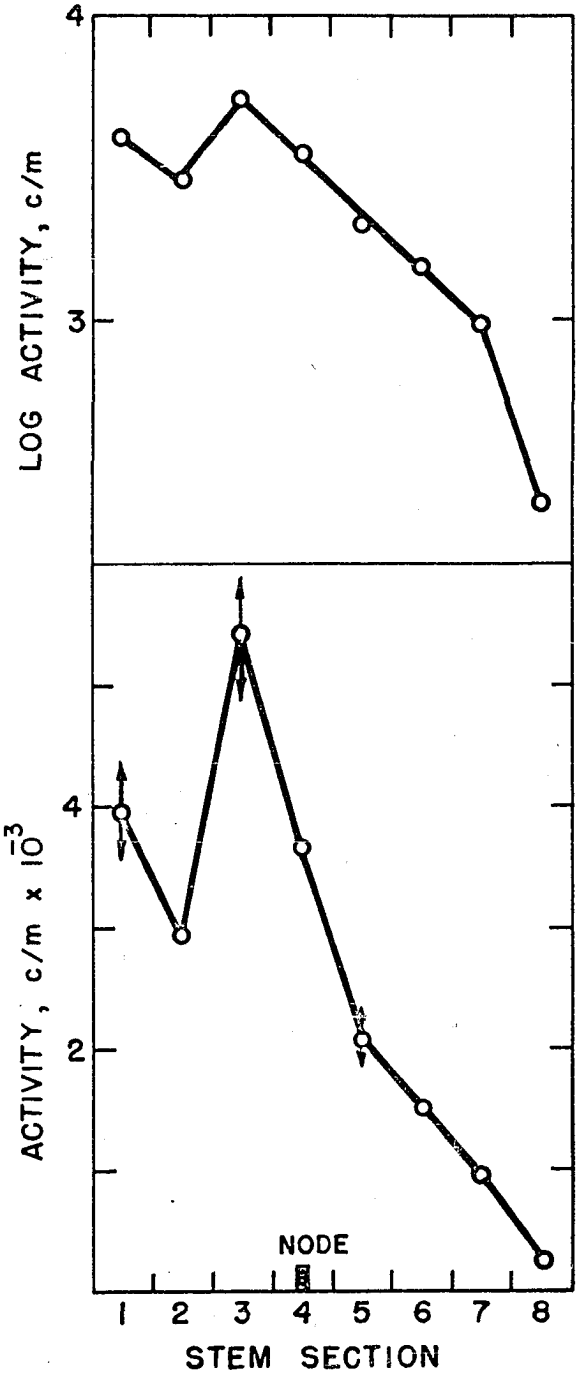


Figure 14. Soybean, total, stem-section, T-photosynthate activities; 15 min light period following 1 hr, dark equilibration with THO-vapor

Figure 15. Cucumber, total, stem-section, T-photosynthate activities; 30 min light period following 1 hr, dark equilibration with THO-vapor

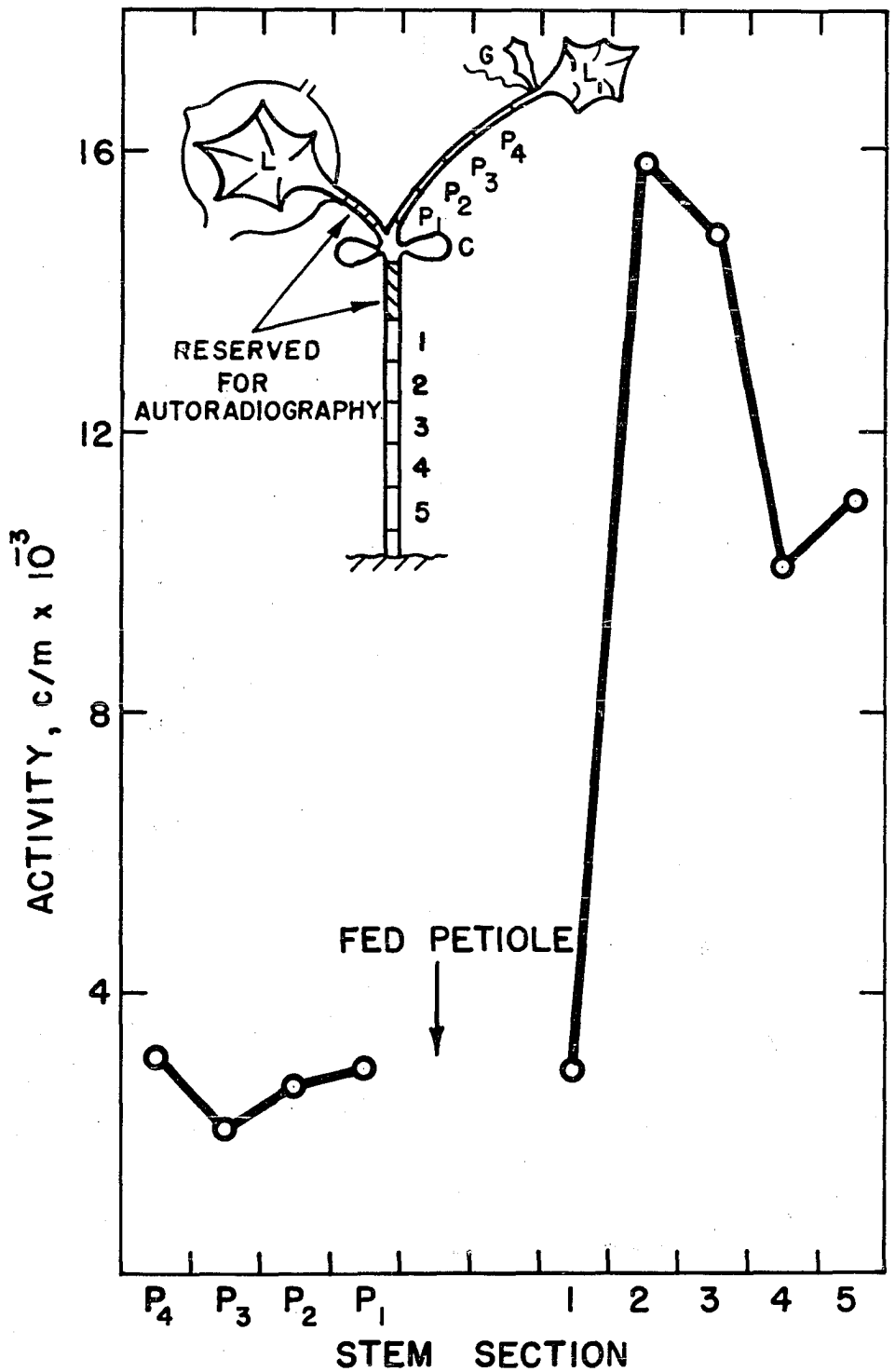

16
12
8
4
ACTIVITY, c/m x 10^{-3}
G
L_1
L
P_4
P_3
P_2
P_1
C
RESERVED
FOR
AUTORADIOGRAPHY
1
2
3
4
5
FED PETIOLE
P_4 P_3 P_2 P_1 1 2 3 4 5
STEM SECTION

Table 10. Cucumber, THO and T-photosynthate distribution; 30 min light period following 1 hr equilibration in the dark with saturated vapor from 10 mc/ml, THO

Section	V[a] (ml)	T-ph. (c/m)	THO (c/m/ml)	% Molar ratio[b] (section/source)	
				T-ph.	THO
L	0.7	875,000	702,000		
L_1	0.6	19,200	22	4	0.003
G	0.1	7,700	27	10	0.004
C	0.3	198	163	0.09	0.02
P_4		3,110			
P_3		2,030			
P_2		2,680	trace		
P_1		2,940	trace		
1	0.2	2,920	8	2	0.001
2		15,800	trace		
3	0.2	14,800	10	10	0.001
4		10,500	trace		
5		11,000	trace		

[a]Volume of extracted water, accurate to ± 0.02 ml.

[b]Calculated assuming that V is a measure of the tissue volume. The fed leaf activity was normalized to 62 percent sucrose, as in Part II.

Discussion

The negative results of Expt. 3,a were taken as an indication that subsequent petiole trials would not yield artifacts caused by internal water tensions. In the 15 minute trial where 50 microcuries per ml, THO was employed (Table 7), detectable amounts of THO were found only in plant sections immediately adjoining the fed petiole; the recovery ratio being about the same as that of the C^{14}-fructose. In the rest of the experiments, the highest available concentrations of THO were employed. Where THO activity was detected, the recovery ratios [except for section P_1 (Table 8) and section C (Table 10)] were two or three orders of magnitude less than those for the other translocates. On this basis, one must conclude that there was no active, aqueous phase in the translocation stream.

It might be reasoned that this conclusion is invalid because of the possibility of irrigation of the transport system by upward moving water of transpiration or guttation. The results of Expt. 3,b (6 minute trial) would appear to be a reasonable argument against this event, on the basis of the following analysis.

At five weeks of age, the vascular tissue between the third and fourth nodes of the soybean stem is well developed. The vascular bundles have merged and the xylem and phloem are well separated by a continuous ring of cambium. The

xylem is in the form of a continuous, irregular sleeve on the inside of the cambium and the phloem cells are isolated in small groups, within the phloem parenchyma, on the outside of the cambium. At this region of the stem, a conservative estimate of the active xylem-cross-section was taken to be 4×10^{-3} cm^2. This included the metaxylem only (the vessels appeared to be open and functional).[1]

It is commonly agreed [see for example (21)] that transpiration rates, in broad-leaved mesophytes, are directly related to leaf area; being, as an upper limit (under the most favorable transpiration conditions), about 2 cm^3 per hour per dm^2 of leaf area, measured on one surface. A generous estimate of the total leaf area for the plant used in the 6 minute trial is 50 cm^2. It would be expected, on this basis, that the maximum possible transpiration rate was 1 cm^3 per hour. Using the above estimate for the xylem cross section, one obtains a maximum linear velocity of 250 cm per hour.

It may be estimated, by extrapolating the linear portion of the semilog plot in Figure 13 to the abscissa intercept, that the velocity of C^{14}-translocate was about 120 cm per hour. Assuming, then, that the THO entered the stem in

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[1]Appreciation is expressed to Mr. J. P. Miksche, Department of Botany and Plant Pathology, Iowa State College, Ames, Iowa for allowing examination of unpublished, prepared, soybean stem material.

comparable amounts with the C^{14}-fructose and that it was removed by the upward moving transpiration stream at the petiole exit, one would anticipate a maximum dilution factor of two in the upper stem sections. As may be seen from Table 8, no THO activity was detected above the entrance petiole which was at section number 4. This argument many be extrapolated to the 15 minute, soybean trial of Expt. 3,c. The THO recovery ratio in the growing point was over 100 times less than the T-photosynthate recovery ratio in the adjoining stem sections, beneath the entrance petiole. It is a safe assumption that the action of maintaining the only trifoliate in a water-saturated atmosphere, reduced the transpiration rate at least ten times. Therefore, there should have been a large accumulation of THO in the growing-point section.

There is further evidence, in the 6 minute experiment, that no such dilution took place because the THO recovery ratio in petiole-section P_2 was less, by two orders of magnitude, than that in P_1 whose end dipped into the radioactive solution. There remains the possibility that guttation took place, outward into the fed solution in Expt. 3,b and into the fed trifoliate, in Expt. 3,c. Plants commonly display this phenomenon under conditions of high atmospheric humidity, presumably, as a result of osmotic work in the roots. However, rates of upward movement during guttation are relatively slow. Although no measurements have been reported on soybeans,

the results obtained with other plants support this contention. Kramer (37b) compared initial transpiration rates with exudation rates that occurred after removal of the tops, in five species (including tomatoe and sunflower) that were maintained in soil at field capacity. In no case did the exudation rates exceed 4 to 5 percent of maximum transpiration rates.

In spite of the evidence, from these experiments, against active participation of water, it is still difficult to understand why so little water appeared with the photosynthate. It is reasonable to expect that there should be considerable water-of-association in the translocate and one is led to wonder if an isotope effect was present. It seems unlikely that there would be discrimination against THO, on the basis of molecular space-charge distribution, that might effect, for example, hydrogen bonding. Likewise discrimination by virtue of isotopic mass difference seems improbable. Wang, et al. (62a) have shown that THO has self-diffusion characteristics that are quite similar to other water species. At 25° C, they obtained diffusion constants (x 10^5 cm^2/sec) of 2.34 for DHO^{16}, 2.44 for THO^{16} and 2.66 for HHO^{18}.

Biddulph and Cory (6) have reported translocation of THO, from the leaves of kidney beans, that would appear to be at variance with the present observations. THO (2,500 microcuries) was applied, in a liquid spray with $NaH_2P^{32}O_2$, to a 1 inch diameter circle on the under surface of a trifoliate

leaflet; and $C^{14}O_2$ was released, simultaneously, to the upper surface over the sprayed area. Although the concentration of THO applied and the THO-leaf-incorporation were not specified, the amount moved per unit activity applied was about 30 times less than P^{32} and about 50 times less than C^{14}. Nevertheless, they obtained THO in stem sections, 6 inches below the entrance node, in concentrations of 50×10^{-12} moles per ml.

They measured THO activity, after initial reduction to tritium gas, in an internal geiger tube. Although their assay method is undoubtedly more sensitive than the one employed in the present experiments, the latter should be capable of detecting this level of activity. 50×10^{-12} moles per ml corresponds to 3×10^{13} atoms of tritium per ml or, to an activity concentration of 1.4 microcuries per ml (approximately 300 c/m, according to Figure 10).

The discrepancy between these investigations appears, therefore, to be real and must arise either from the method of tracer application or, from differences in hydrostatic conditions between the two plant systems. The latter seems to be a possibility. In all of their experiments, Biddulph and Cory (6) found that the front of the THO activity contained a relatively large amount of tracer. They were careful to explain that this could be an indication that the application of tracer water to the epidermis of the leaf affected a local reduction of tension, at that spot, which

facilitated the movement. In the case of the present investigation, any such tensions would have been relieved by the initial equilibration with pure water in the petiole experiments and by uniform addition of water, in the leaf-vapor experiments.

PART IV. AUTORADIOGRAPHY WITH TRITIUM-PHOTOSYNTHATE

An important parameter of photosynthate translocation is the precise geometry of its cross section. Although it is commonly assumed that the phloem, sieve elements form the conducting pipes, the only direct supporting evidence has come from autoradiography. Biddulph's autoradiograms of kidney bean stems (8) show general localization of foliarly applied P^{32} and S^{35}, in the phloem region. Thaine and Walters (58) reported only preliminary details of their work with C^{14}-photosynthate in soybeans.

The application of autoradiography to labelled photosynthate presents special difficulties. Because photosynthetic compounds are water-soluble, one must attempt to prepare (and expose on emulsion) histological sections, in the dehydrated state. Moreover, in attempting to locate the source of radioactivity, in cells 5 to 10 μ in diameter, the resolution is limited by the range of the emission product. Even the relatively soft C^{14} beta, for example, with maximum energy at 156 Kev has an average range, in emulsion, of about 20 μ [Fitzgerald (27a)] ; and may leave a tortuous track of exposed silver grains behind it.

It was thought worthwhile, therefore, to attempt to adopt the autoradiography technique to stem tissue containing tritium-labelled translocate. The extremely weak H^3 betas (17.9 Kev maximum) have ranges of only 1 to 2 μ.

Experimental

Tissue lyophilization and imbedding

Petiole and stem material from plants whose leaves had been fed THO vapor, as in Part III, were lyophilized in a Pyrex freeze-drying apparatus which is illustrated in Figure 16,a. This was identical in design to that described by its inventor, Jenson (35, 36), except for the tissue holder shown in Figure 16,c, and the drying procedure was very similar to that described by him.

The tissue holder was made from brass and was designed so that tissue drying and imbedding could be accomplished with a minimum of tissue manipulation. It consisted of two matching disks that were drilled to accommodate eight, half inch, brass-tube compartments. When assembled, the disks were separated by the circular piece of brass mesh and close alignment was ensured by two soldered pins in the lower plate, protruding into matching holes, in the upper plate. The upper brass stud held the assembly together and it, in turn was threaded to accomodate the Teflon rod. Each compartment had a closely-fitted, brass-mesh cap.

The tissue preparation and lyophilization was accomplished in the following steps.

a) The Jenson drier, without tissue holder, was connected to its separate pumping system (Figure 17) and was immersed in the large Dewar containing a propylene glycol methyl

ether, coolant (Dowanol 33B, obtained from Dow Chemical Company). To this was added small chips of dry ice until the temperature was reduced to about -60° C.

b) The assembled tissue holder was immersed, almost to the tops of the compartments, in a beaker containing liquid, n-pentane which was cooled to its freezing point (about -130° C) by a surrounding, liquid-nitrogen bath.

c) The fresh tissue was rapidly sectioned, with a razor, into 1 - 2 mm lengths and these were immediately lowered into the individual compartments. The n-pentane was stirred constantly during this period to insure rapid transfer of heat from the tissue.

d) The compartment caps were secured and the tissue holder was quickly lowered into the cooled drier so that it rested on its rubber "O" ring at the bottom. The brass lid, with its "O" ring, vacuum seal was lowered in place.

e) Pumping was started immediately, with the metering valve closed (V_2, in Figure 17), until the pressure indicated by the manometer was about 1 cm, Hg. Then dried nitrogen gas, at 1 - 2 lb above atmospheric pressure, was bled into the system via V_2. Following Jenson's recommendations (36) the pressure was maintained at 1.5 ± 0.1 cm, Hg during the drying run. The dry, cooled gas passed upward over the tissue at a rate of about half a mole per hour. The Dowanol 33B coolant proved to be very satisfactory. The dry ice chips remained at the surface and the cooled, upper liquid layers

Figure 16. Tissue drying and imbedding apparatus

a. Jenson drier, b. imbedding chamber,
c. tissue holder.

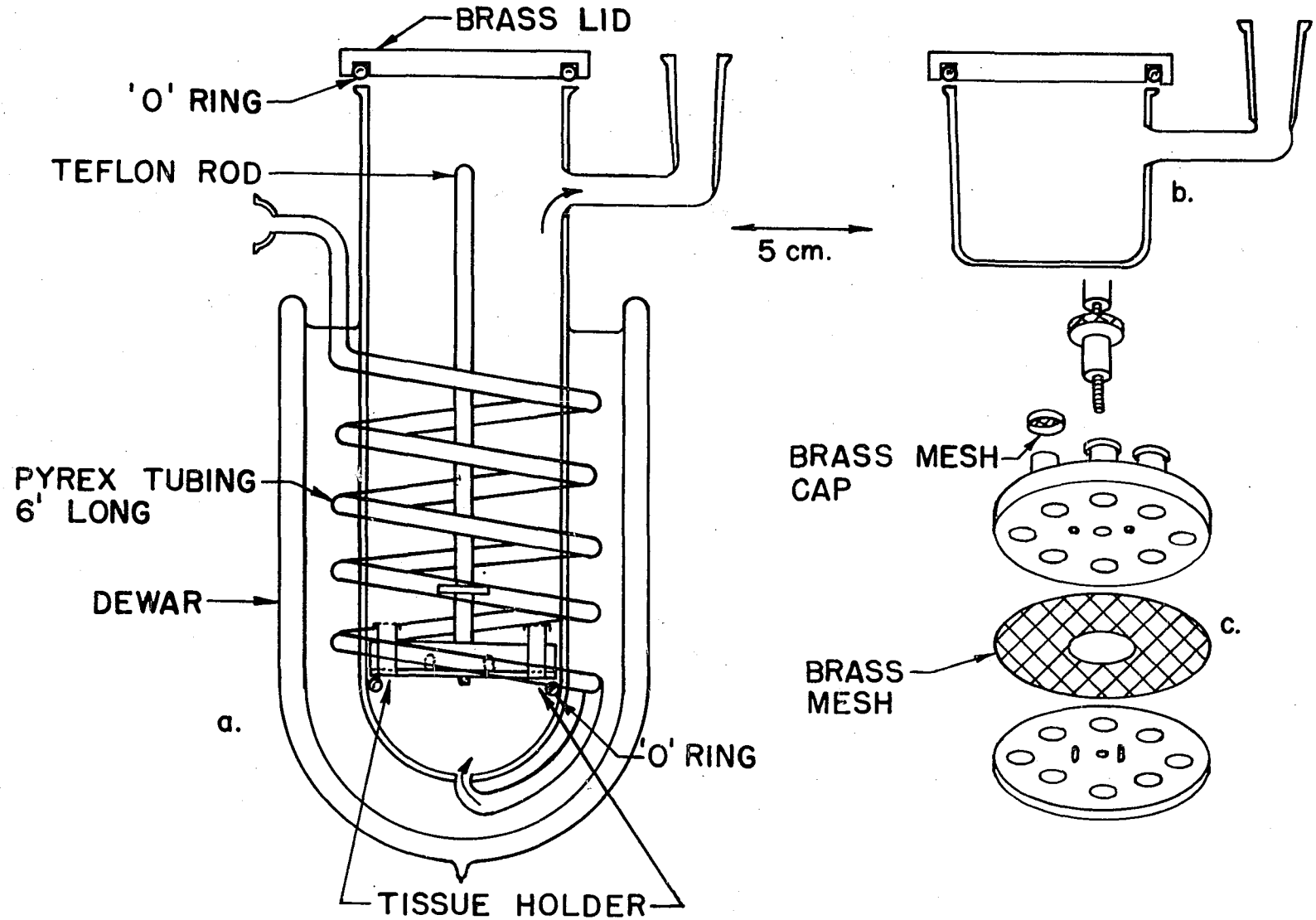

BRASS LID
'O' RING
TEFLON ROD
PYREX TUBING
6' LONG
DEWAR
a.
'O' RING
TISSUE HOLDER
5 cm.
b.
BRASS MESH
CAP
BRASS
MESH
c.

Figure 17. Vacuum systems for lyophilization and imbedding (separated by the central stopcock)

N nitrogen cylinder

V_1 cylinder, regulator valve

D anhydrous $Mg(ClO_4)_2$, drying agent

V_2 metering valve

t_1 low-temperature thermometer

T tissue drier

M closed-end, mercury manometer

P_1 vacuum pump. Welch, two-stage, Duo-seal, 1,400 series

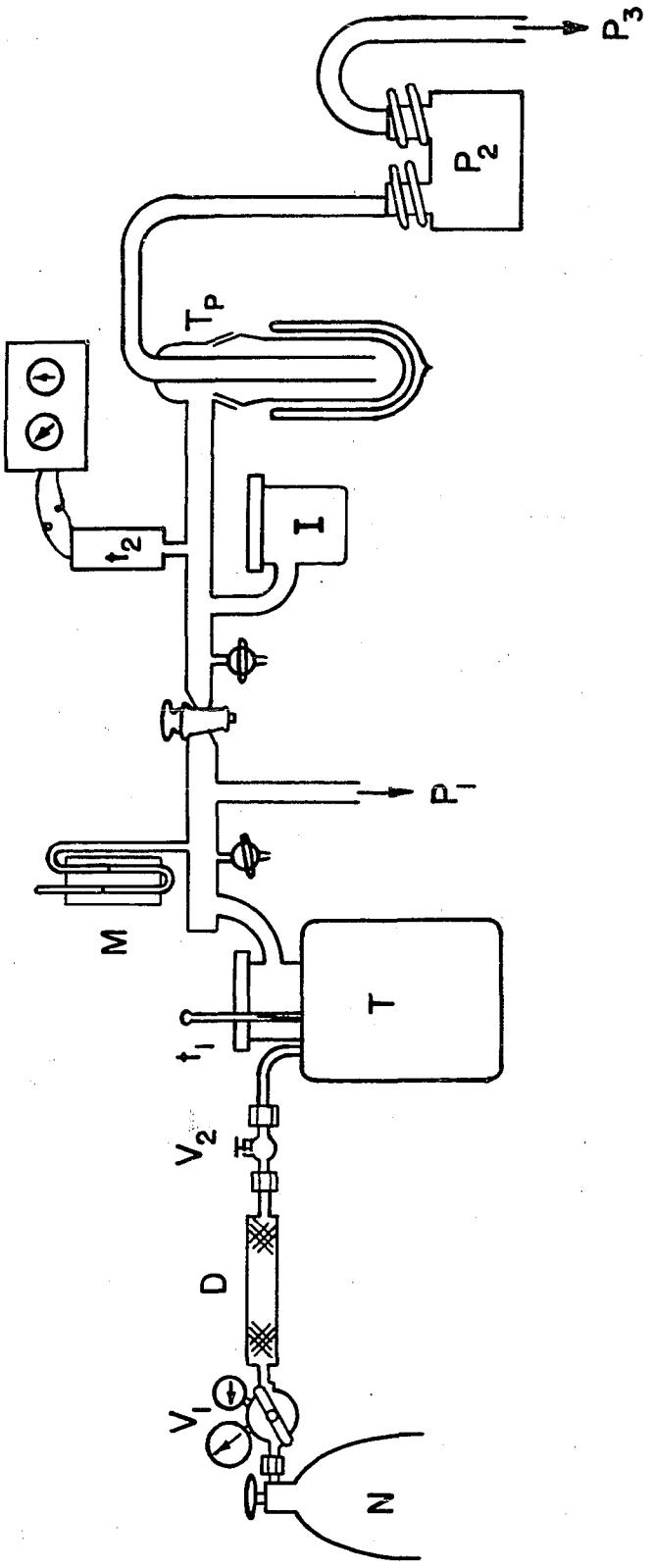
t_2 thermocouple, vacuum gauge

I imbedding chamber

T_p liquid-nitrogen trap

P_2 oil-diffusion pump

P_3 vacuum pump. Welch, two-stage, Duo-seal, 1,405 series


P3
P2
Tp
t2
I
P−
M
T
t1
V2
D
V1
N

were continually replaced by convection. Gas bubbles were released constantly throughout the coolant and the bath temperature varied less than 3° C, from top to bottom so that no mechanical mixing was required.

Jenson (36) recommended a drying period of 6 - 8 hours, his criterion for dryness being the imbedding properties of the tissue. The period decided upon for the present application was chosen, after several trials, for convenience at 16 hours. Drying was commenced at about 4:00 p. m. and the bath temperature was regulated until midnight. The gas system was then left running overnight (allowing gradual equilibration with room temperature) and imbedding was commenced the next morning.

Vacuum infiltration was done in the following sequence. a) The imbedding chamber, shown in Figure 16,b, was connected to its separate vacuum system which contained a liquid-nitrogen trap, an oil diffusion pump and a high-capacity vacuum pump (Figure 17). Histological wax (Tissuemat 50 - 54° C melting point, Fisher Scientific Company) was added, the vacuum lid secured and the imbedding vessel was immersed in a water bath which was held at 60° C. The system was pumped down to about 5 μ, Hg and degassing and melting of the wax proceeded over a period of about 4 hours. The wax was considered to be degassed when no further bubbles appeared at a pressure of 1 μ, Hg.

b) Simultaneously with step a), the tissue-drier Dewar (the nitrogen was still flowing) was replaced with a water bath and its temperature was gradually raised, over about 1 hour, to the wax temperature.

c) Both vacuum systems were broken, the tissue chamber was transferred quickly and immersed in the molten wax and the entire system was again degassed (usually requiring about 1 hour).

d) The vacuum was released slowly over a period of about half an hour and the system was allowed to cool to room temperature.

e) Excess wax was removed from the solid cast and the tissue holder was broken open by separating the brass disks. The tissue compartments were gently punched out and the tissue was blocked for sectioning.

Considerable difficulty was encountered in obtaining adequate, rapid infiltration of stem and petiole material with this technique. The following unsuccessful variations were attempted: waxes of different manufacture and melting points; the drying period was varied from 4 to 36 hours; individual degassing of wax and tissue prior to immersion.

Material from the following species was tested: soybean, Glycine max; common bean, Phaseolus vulgaris; squash Cucurbita moschata duchesne; cucumber, Cucurbita sativus. The last proved to be the most successful, about one in three

sections being sufficiently infiltrated to give acceptable histological sections.

Autoradiography

The techniques described herein, except for minor refinements in the mounting method, are attributable to a number of investigators. They are well reviewed by Fitzgerald (27a).

Emulsion All manipulations with undeveloped emulsions were carried out in a photographic dark room using a Wratten-2 filter (with a 15 watt lamp) at a working distance of 4 feet. It was found necessary to adopt well-defined routines to guard against fogging artifacts. One by three inch, histological slides were subbed with a 0.5 percent gelatin, 0.05 percent chrome alum solution and air dried. Kodak, Autoradiographic, Permeable Base, Stripping Film was cut into 1 x 3.5 cm rectangles. The loosely-bound emulsion and gelatin base was slowly stripped from the cellulose acetate base by grasping one corner with stainless steel forceps. It was necessary to breathe on the emulsion and earth one wrist to a water pipe, in order to prevent static discharge during the stripping. The emulsion and base were floated, emulsion side up, on distilled water at about 25° C for 3 minutes at which time, it had about doubled its original area. A subbed slide was slipped under it and was raised

gently so that the emulsion fell smoothly over the surface and the excess draped itself over the slide edges. The prepared slide was air-dried for an hour after which, it was stored in a new, stainless steel developing tank containing a small quantity of $CaCl_2$ desiccant. Slides prepared in this fashion provided a smooth emulsion surface of about 2 μ thickness that adhered well to the glass slide (see Figure 18,c).

Sectioning and mounting Acceptable histological sections were microtomed at 10 μ. The following unsuccessful, dry-mounting methods were attempted: mechanically-stretched, paraffin sections were sandwiched between the emulsion and a second slide (sections were lost during processing); paraffin sections were floated on a clean mercury surface at 50° C and the emulsion surface was pressed onto the section (sections were lost during processing and the emulsion was fogged). Finally, individual 8 μ sections were cut by coating the face of the paraffin block with molten wax between each stroke of the microtome blade (Figure 18,a) and coating the latter with a thin layer of Silicon grease. About one in four sections, prepared in this way, were useful. These were removed to the dark room and placed, four at a time with sections up, on the rubber-stopper mounting block shown in Figure 18,b. They were softened briefly by radiation from a histological, hot-plate and the emulsion slide was pressed downward over the

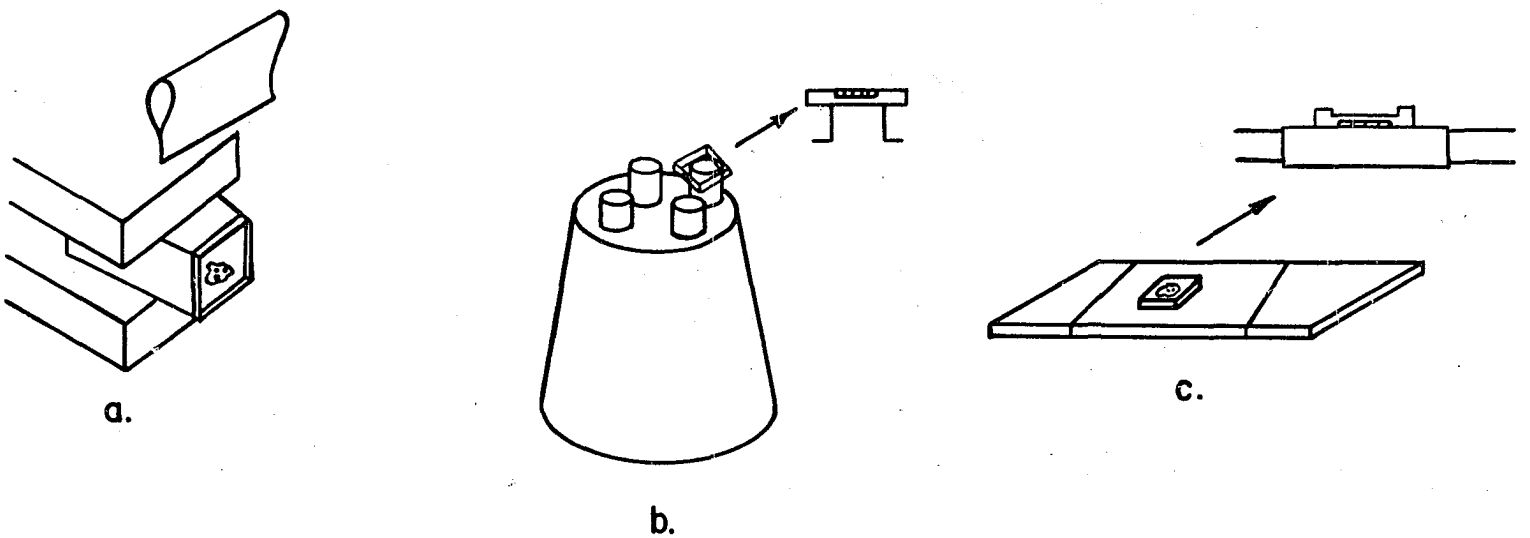


Figure 18. Autoradiogram sectioning and mounting techniques

a. Microtome block with supporting paraffin layer, b. rubber-stopper, mounting block, c. prepared autoradiogram.

sections. Dry mounting in this fashion also proved unsatisfactory as did similar attempts after equilibration of the emulsion in a moist atmosphere. The final method was to apply the lightest possible layer of egg albumen to the emulsion surface before pressing it onto the sections.

Exposure and processing The autoradiograms were stored during exposure at about 5°C in sealed, black, plastic slide-boxes that contained a small quantity of $CaCl_2$ dessiccant. The processing was done horizontally in flat staining jars that were maintained in a shallow bath in which water was circulated continuously at 20 ± 1° C. The processing routine is detailed in Table 11. At step 17, the excess emulsion around the sections was removed with a razor. After this step also, the developed, stained autoradiograms were dehydrated by running them backwards, from step 10 to step 2; and a cover slip was secured over the section and emulsion with synthetic resin.

Results and Discussion

The cucumber plant described in Part III, Expt. 3,c, was sectioned as is indicated in Figure 15 (page 70). The primary-leaf-petiole and the stem section immediately beneath the cotyledons were further sectioned into 1 - 2 mm lengths, lyophilized, vacuum-imbedded and autoradiographed.

Table 11. Autoradiogram processing schedule

Step	Reagent	Time (minutes)
1	xylene I, dewax	60-90
2	xylene II	2
3	xylene III	2
4	absolute ethanol I	2
5	absolute ethanol II	2
6	95% ethanol	2
7	70% ethanol	2
8	50% ethanol	2
9	30% ethanol	2
10	distilled water	2
11	Kodak, D-19 developer	2 (gentle agitation)
12	distilled water	30 seconds
13	Kodak, F-5 fixer	4
14	gently-flowing tap water	30
15	Mayer's hemalum, histological stain	5
16	1% HCl, destain	10-15 seconds
17	gently-flowing tap water	30

Five autoradiograms each (4 sections per slide) were prepared of petiole cross-sections, petiole longitudinal-sections, stem cross-sections and stem longitudinal-sections. Because of the uncertainties associated with microtomy, mapping of the longitudinal-sections was not possible. Autoradiograms were exposed in the same storage box with blanks (4 stem and 4 petiole) from tissue treated in the same way except that it was from an identical, nonradioactive plant.

An accurate forecast of the necessary exposure time was not possible because of the unknown tissue, activity concentration. Biddulph (8) obtained adequate exposure with P^{32} and S^{35}, in 55 days, from 15 μ kidney bean sections that indicated 20 disintegrations per minute in an internal flow counter. Similar checks with the tritiated tissue gave 25 - 30 net counts per minute and the first stem and petiole cross-sections were processed after 14 days because of the lesser counting efficiency for tritium. The petiole sections washed away during processing. In one stem section there appeared to be a few areas of localized, exposed, emulsion grains, just distinguishable from background. Therefore, the remainder of the slides were processed at from 80 - 90 days.

The results, in general, were disappointing. In spite of extreme care during processing, many sections were either displaced from their initial position on the emulsion or were lost entirely. The critical point in the processing appeared to be the first step (Table 11) which was necessary in order to remove the upper paraffin layer (Figure 18,c). Four slides were rejected because of heavy accumulations of developed grains appearing in criss-cross lines in the emulsion. These were thought to result from stresses occurring in the emulsion during exposure.

Table 12 lists the slides and sections which survived processing together with the number of sections where grain density could be attributed to radioactivity (column R).

Table 12. Useful autoradiograms

Quantity and type	Sections surviving processing	R
2 petiole cross.	8	1
1 petiole cross. blank	4	
4 petiole long.	11	5
1 petiole long. blank	4	
1 stem cross.	4	(14 day exposure) 1
3 stem long.	8	6
1 stem long. blank	3	

In all sections where grain density was attributed to radioactivity, except one (Figure 20), the grains were scattered diffusely beneath vascular tissue having their greatest density under sieve and companion cells. Also, in these cases, grain lines appeared, throughout the sections, beneath cell walls. These were fortunate artifacts (they also appeared in blanks) because they served as criteria for section alignment and contact with the emulsion. Figure 19 is a photomicrograph of a typical longitudinal section.

The one useful petiole cross-section contained well localized activity in two adjacent vascular bundles one of which, is shown in Figure 20. Intense grain-density may be seen beneath the most-recently-differentiated phloem. Greatest density appears to be associated with the companion cells.

The overall results add convincing evidence to the notion that the phloem region, in general, is the tissue through which photosynthate moves. One autoradiogram appears to provide additional information regarding the specific phloem tissue that was involved in at least two vascular bundles of the petiole. The difference between the two types of localization makes one suspicious that the albumen adhesive was the cause of considerable diffusion and that perhaps the better localization of Figure 20 resulted fortuitously from a portion of tissue that remained in contact with the emulsion, without adhesive.

Figure 19. Autoradiogram of T-photosynthate in cucumber, petiole longitudinal-section

Primary leaf saturated with vapor from 10 mc/ml THO, one hour in the dark and 30 minutes in the light. Sections lyophylized, pressed on stripping film with albumen adhesive, 90 day exposure. Microphotographed in bright-field (x 420).

a. Tissue level.

b. Emulsion level showing activity scattered diffusely in vascular tissue with greatest density under sieve and companion cells. The grain lines along cell walls are taken to be pressure artifacts.

a.

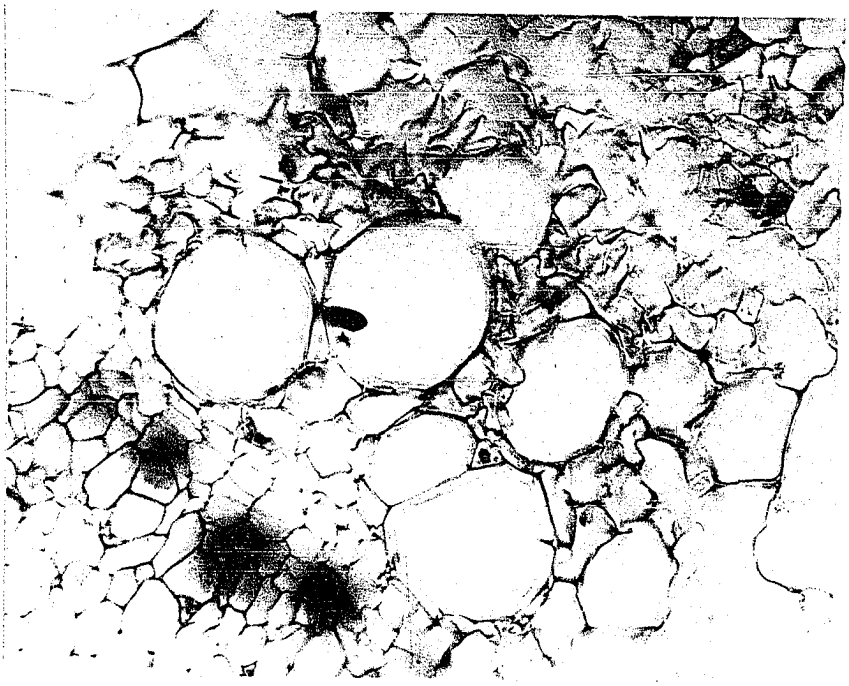
b.

Figure 20. Autoradiograph of T-photosynthate in cucumber, petiole cross-section

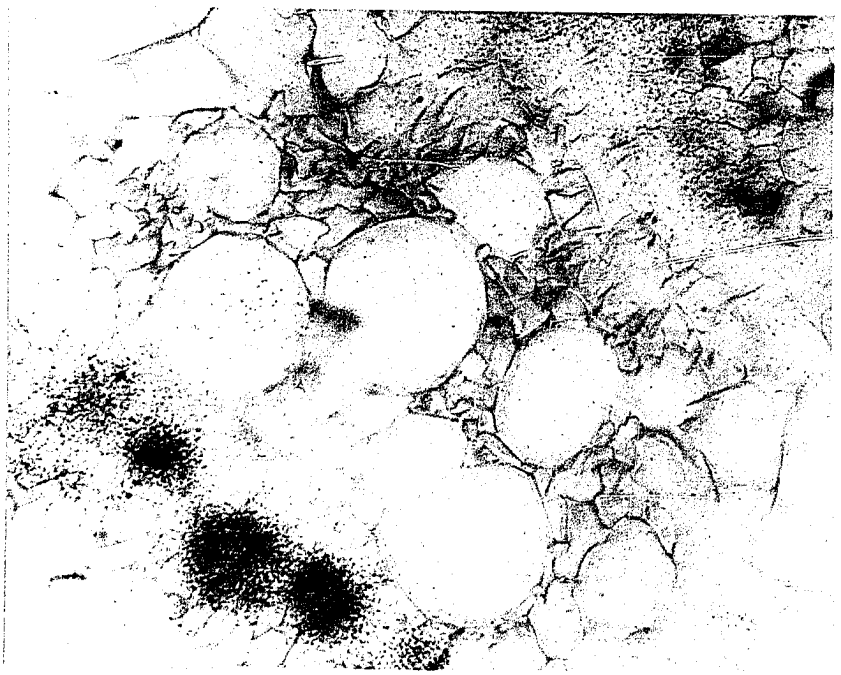
Primary leaf saturated with vapor from 10 mc/ml THO, one hour in the dark and 30 minutes in the light. Sections lyophylized, pressed on stripping film with albumen adhesive, 87 day exposure. Microphotographed in bright-field (x 420).

a. Tissue level.

b. Emulsion level showing localized activity in youngest sieve and companion cells.



a.



b.

One further disturbing aspect may be seen from the gross analysis in Figure 15. At stem section number 2, a large increase in total stem activity may be seen. If it is speculated that this large peak of activity represents the main, moving source of translocate which passed through the autoradiographed tissue, at some earlier time, then one might expect to find only its "diffusion-trail" at higher levels in the stem and petiole.

GENERAL DISCUSSION

From the rough tactics employed in chopping and steaming plants in Part I, it appears that photosynthate translocation, during short periods, does not occur as a result of immediate growth requirements. Neither is it moved because of osmotic potential energy at the source of supply. The anomalous, directing influence of the root, suggested by the stem-cutting and steaming experiments, indicates that some locus below the epicotyl may be the immediate "power supply". The retardation observed by Nelson and Gorham (49) after chilling of roots might also be interpreted in this way.

The similarity in chloride and photosynthate translocation, found in Part II, is thought to add support to the concepts of mass flow of solutes in the translocation channels. The application to the model borrowed from Horwitz (31) is fraught with assumptions and extrapolations and on the basis of only one simultaneous, Cl^{36} - C^{14} experiment is presented with apology.

The pressure-flow hypothesis as originally conceived by Munch (46) can no longer be considered valid if the THO measurements of Part III are meaningful. It is hoped that the significant differences between these measurements and those of Biddulph and Cory (6) may be resolved by comparisons of different species using identical feeding and assay tactics.

The tritium autoradiography in Part IV must be considered to be only of a preliminary nature and adds to the present knowledge only in support of the popular supposition that the phloem is the translocation route. It is hoped that the techniques described herein may provide assistance in further work with T-photosynthate using more elegant methods.

In every stem activity analysis, the orderly distributions were disrupted, about midway down the stem, by activity peaks (Figures 3, 4, 6, 7, 8, 12, 13, 14 and 15). Similar variations are evident from the data of other workers [for example Vernon (60) Biddulph and Cory (6)]. Some have been attributed to accumulations at stem nodes. In the present experiments, an activity peak occurred at a node in one experiment only (Figure 4) and it is concluded that these localized centers of activity must have some meaning in the translocation mechanism. In three cases, besides the data of Figure 8, namely Figures 3, 13 and 14, there are suggestions that the peaks occur between descending exponential plateaus of different level. It is felt that these add impetus to the concept of the second source depicted in Figure 21; regardless of the acceptability of that analysis which was designed to show similar modes of transport for C^{14} and Cl^{36}. Whether "S_2" was moving or stationary is, of course, unknown. It must be admitted that extension of such a concept to encompass translocation, in general, would have little meaning at this time. There are many unanswered questions associated with

these short-time experiments. For example, do they represent the steady state and, indeed, is there such a thing as steady-state translocation?

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APPENDIX

The Horwitz model for "flow through a pipe with irreversible loss through the walls" (31) is summarized as follows. Translocation is visualized as unidirectional mass flow of the fluid contents of a pipe, regardless of the actual mechanism. Solute may diffuse outward through the pipe walls but no net transfer of water is permitted. Sufficient mixing within the pipe occurs to provide a uniform concentration throughout its cross-section. Attenuation of the concentration at the front of flow of the solute is considered unimportant. For short-term translocation, consider the following situation as interpreted in the diagram of Figure 21.

Suppose for a distance h somewhere along the pipe, it is surrounded by a steady state concentration C_1 of the radioactive material X, and that K is a first order constant characterizing the rate of transfer of X through the pipe wall in the region h. If the velocity of flow of the fluid in the pipe is v and the pipe cross sectional area is $A_=$, then the concentration of X in the pipe at the outflow edge of the region h is

$$C_0 = C_1(1 - e^{-\frac{Kh}{A_p v}}) \qquad (1)$$

In order to account for X after it leaves the region h, the following additional symbols are used:

x = distance along the pipe from the region h in the direction of flow

t = time.

C_p = concentration of X in the pipe at any distance x.

C_t = concentration of X in the tissue surrounding the pipe at any distance x.

k = constant for diffusion of X through the pipe wall or a first order chemical reaction rate constant for removal of X from the flowing stream into a bound form.

A_t = cross sectional area of tissue surrounding the pipe.

C_p will be subject to the following relation:

$$\frac{dC_p}{dx} = - \frac{k}{A_p v} C_p = - \frac{k_1}{v} C_p \ . \qquad (2)$$

Integrated, this gives:

$$C_p = C_0 e^{- \frac{k_1 x}{v}} \ . \qquad (3)$$

The concentration, C_p, in the pipe, therefore, is not time dependent, and fulfills equation 3 from the end of region h to the front of flow of X. Beyond that it is zero.

Therefore, the concentration, C_t, (which is in a bound form), in the tissue surrounding the pipe at a distance x, anywhere from region h to the front of flow of X, is given by:

$$C_t = \frac{k}{A_t} C_0 e^{-\frac{k_1 x}{v}\left(t - \frac{x}{v}\right)} = k_2 C_0 e^{-\frac{k_1 x}{v}\left(t - \frac{x}{v}\right)} . \qquad (4)$$

The total amount of X, then, at any level in the stem from region h to the front of flow is

$$X = A_p C_0 e^{-\frac{k_1 x}{v}} + A_t t k_2 C_0 e^{-\frac{k_1 x}{v}} - A_t \frac{x}{v} k_2 C_0 e^{-\frac{k_1 x}{v}} , \qquad (5)$$

or

$$X = C_0 e^{-\frac{k_1 x}{v}} \left\{ A_p + k \left(t - \frac{x}{v} \right) \right\} , \qquad (6)$$

and

$$\log_e X = \log_e C_0 + \log_e \left\{ A_p + k \left(t - \frac{x}{v} \right) \right\} - \frac{k_1 x}{v} . \qquad (7)$$

The slope of the curve relating $\log_e X$ and x is

$$\frac{d \log_e X}{dx} = - \frac{k}{v \left\{ A_p + k \left(t - \frac{x}{v} \right) \right\}} - \frac{k}{v A_p} , \qquad (8)$$

Horwitz (31) then applied the following parameters thought to be applicable to the kidney bean plants used by Biddulph

and Cory (6): A_p = 0.1 mm^2, v = 50 cm per hour and k = 2 mm^2 per hour (the latter being the accepted value for sucrose in water). He plotted equation 6 on semilog co-ordinates and obtained curves which were very similar to the experimental curves of Biddulph and Cory (6); for P^{32} application to leaves in 15 and 30 minute translocations. The plots were descending lines of almost constant slope, the 15 minute one increasing its negative slope slightly toward the lower stem sections.

The slope of such a semilog plot, as is seen from equation 8, depends upon k, A_p and v. When two tracers are applied simultaneously to the same plant, similarity in the semilog slopes cannot be construed to mean similar modes of transport without fixing at least two of the above parameters.

The results of Expt. 2,c are replotted on the lower graph of Figure 21 and a second theoretical source S_2 has been inserted to encompass roughly stem sections 2, 3 and 4. The contributions of S_1 and S_2 to the total activities are indicated by the broken-line curves and they were arrived at graphically in the following manner.

The semilog plot of Figure 8 was expanded in Figure 22. The points for both tracers, lying outside the discontinuities, were fitted to a common slope (solid lines). S_1 was assigned to the first two experimental points of each tracer and its contributions to the lower stem, obtained by the dotted-line extrapolations. To the right of the maxima, the

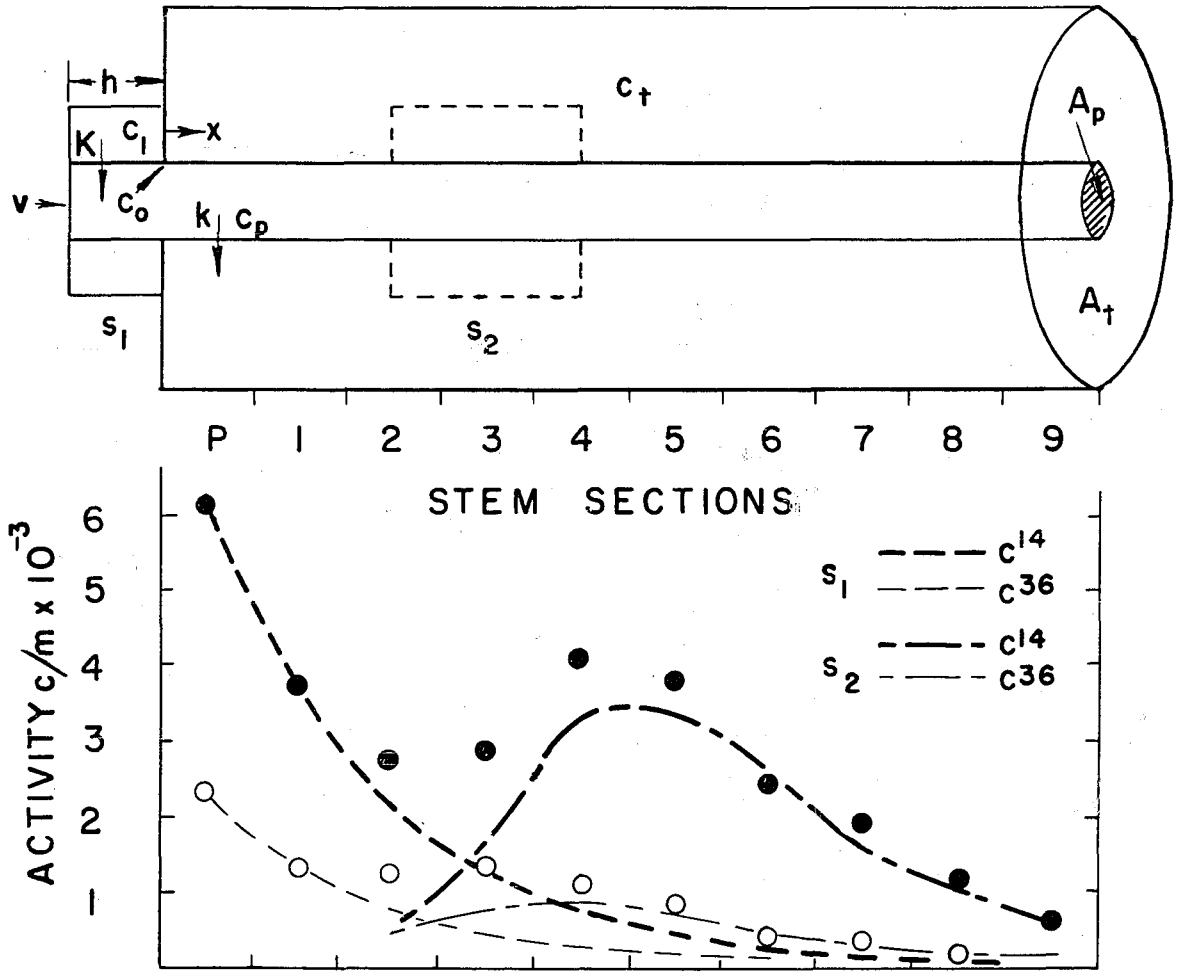

Figure 21. Upper: Horwitz model

Lower: Expt. 2,c reconstructed to show individual contributions of two theoretical sources to the total C^{14} and Cl^{36} stem-section activities

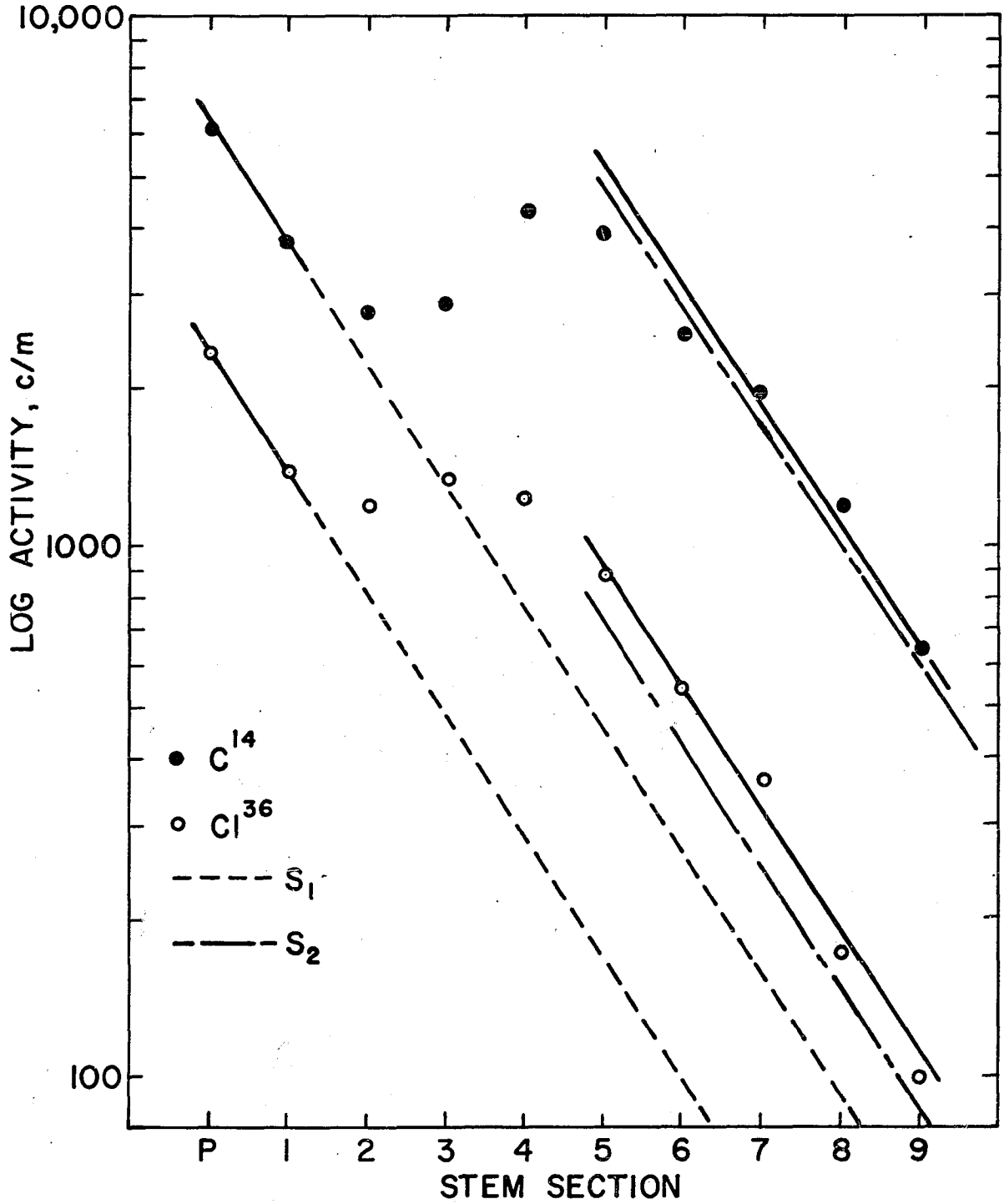

Figure 22. Method of graphical construction for Figure 21

contributions of S_2 (dot-dash lines) were obtained by linear subtraction of the extrapolated S_1 lines from the solid lines. In Figure 21, the contributions of S_1 are, again, the exponential decay extrapolations of the first two sets of points. The S_2 curves were plotted from the dot-dash lines of Figure 22 except for stem sections 2 to 5 where the S_1 values were subtracted from the experimental points.